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ENHANCED PHOSPHORUS SOLUBILITY DURING ANAEROBIC DIGESTION

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Abstract

Worldwide phosphorus (P) consumption has increased massively over the last century, to overcome soil nutrient constraints, and to feed an increasing population with an increasing level of urbanization. However, current P reserves are limited and are expected to deplete in 50-120 years. Hence, new techniques to source P from alternative resources are essential. Waste streams are the largest alternative resource of P, with much of the organic wastes containing P treated by anaerobic digestion (AD). However, P recovery from the digested waste streams is limited by in-reactor P precipitation, and inaccessibility of soil available P in sludge biosolids. In-reactor P precipitation increases the operational costs of a treatment facility due to scaling and precipitation, there are strong advantages in being able to recover P as a purified mineral product. In such circumstances, keeping high PO_4 concentration during AD is vital to enhance its recovery post AD.

This thesis aims to assess two different techniques to enhance PO_4 concentration during AD from waste activated sludge (WAS): low pH and high pressure (enabling low pH). Low pH single stage AD was tested in both batch and continuous mode. In the batch study, biochemical methane potential tests were conducted for 51 days at a pH range of 5.0 to 7.2 in two separate sets (two different WAS samples collected from municipal WWTP). Low pH (< 5.7) caused a significant loss in the methane potential (B_0) of up to 33%, with 3.6 times increase in PO_4 concentration compared to the neutral pH (7 – 7.7), but with no major change in methane production rate coefficient (k_{hyd}). The loss in methane yield was mainly due to decrease in hydrolytic capability rather than inhibition of methanogenesis with volatile fatty acids (VFAs) being < 300 mgCOD L^{-1} and soluble COD < 1300 mgCOD L^{-1} even at low pH. While pH did not influence the acetoclastic community (Methanosaeta dominated), it was the primary driver for the remaining community, and caused a loss of diversity and shift to Clostridia. To validate the results from batch conditions, continuous low pH AD was performed using similar substrate and pH conditions. The influence of the pH on PO_4 concentration was similar in continuous and batch. It was found that the low pH (5.5) caused a significant increase in PO_4 concentration up to 79% of the total P, while methane yield was reduced by 50%. VFAs and SCOD concentrations increased from 40 to 504 mg L^{-1} and 600 to 2017 mg L^{-1} respectively, as the pH was reduced from 7.0 to 5.5. Higher concentration of propionic acid (370 – 430 mg L^{-1}) was recorded at low pH (< 5.5). The reduction in methane yield was associated with a shift in microbial community and decreased destruction of particulate organics. Acidogens dominated at low pH (< 6.0), while methanogens decreased by 88% at pH 5.5 compared to neutral pH. Apart from the loss in methanogenic and hydrolytic capacity, continuous acid dosing to maintain low pH condition was identified as a key limitation with this technology. To assess an alternative method to avoid acid dosing, operation under pressure was assessed (at 1, 2, 4 and 6 bar absolute pressure). The average PO_4 concentration

increased to 51.2 ± 0.01, 56.4 ± 0.05, 65.4 ± 0.1, and 75.3 ± 0.05% at 1 (control), 2, 4, and 6 bar respectively. The specific methane yield was 66.8 ± 3.6, 47.4 ± 4, and 58.5 ± 3.5 L-CH_4 kg-VS_{fed}^{-1} at 2, 4, and 6 bar respectively (averaging 40% increase compared to 1 bar), but VSD and COD removal was unaffected, indicating better gas capture. Total VFAs concentration were below 15 mg L^{-1} at all conditions. The CO_2 content were 27.6, 19.8, 16.7 and 13.5% at 1 (control), 2, 4, and 6 bar respectively (with the balance being methane). Increased pressure caused a substantial change in Archaeal populations, to novel clades, without substantial change in function. Increased PO_4 concentration at high pressure was due to the combined effect of low pH conditions and dissociation of PO_4 based precipitants caused by increased ion activity. Overall, auto generative high-pressure AD is a chemical free technique to improve PO_4 concentration and methane content in the biogas with the main barrier being increased capital cost. Low pH (up to 5.5) and high pressure (up to 6 bar) AD is recommendable to enhance P recovery, where low pH AD can be integrated without changing current infrastructure, while AD at a pressure up to 6 bar may require specialized reactor design.

Declaration by author

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Publications during candidature

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C.M. Mehta	Designed experiments (20%) Wrote and edited paper (20%)
D.J. Batstone	Designed experiments (20%) Wrote and edited paper (10%)

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Keywords

phosphorus solubility, anaerobic digestion, low pH, high pressure, methane production, DNA, solids removal, VFA, COD

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List of abbreviations

Acronyms

AD	anaerobic digestion
ADS	anaerobic digested sludge
Al	aluminum
BMP	biochemical methane potential
Bt	billion tons
Ca	calcium
CA	chelating agents
CH_4	methane
CO_2	carbon dioxide
COD	chemical oxygen demand
DNA	Deoxyribonucleic acid
EDTA	ethylene diamine tetra acetic acid
Fe	iron
FIA	flow injection analyzer
GC	Gas chromatograph
H_2	hydrogen
H_2CO_3	carbonic acid
HiPAD	high pressure anaerobic digestion
HRT	hydraulic retention time
ICP	inductivity coupled plazma
IER	ion exchange resins
Mg	magnesium
mmol	milli mole
Mt	million tons
NH4-N	ammonia
NTA	nitrilotriacetate
OLR	organic loading rate
OTU	Operational taxonomic units
P	Phosphorus
PO_4	Phosphate
SCOD	Soluble chemical oxygen demand

TL	Trillion litres
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

Nomenclature

B_0	Degradability (L-CH_4 g-VS_{fed}^{-1})
k_{hyd}	Hydrolysis coefficient (d^{-1})
Q	Flow rate

1. LITERATURE REVIEW

This chapter develops research motivation from a literature review relevant to the topic. It reviews phosphorus in the environment, and its forms in waste streams. Knowledge of the transformation of phosphorus during anaerobic digestion is also part of this chapter. Key factors influencing phosphorus solubility were investigated for the engineered application of anaerobic digestion. Finally, research objectives and scope of the study are projected based on the problems raised in the literature review.

1.1 Research motivation

Phosphorus (P) is a key nutrient for plant growth, and there is no substitute for P in agriculture (Prud'homme 2015). On a yearly basis, more than 160 million tons (Mt) of rock phosphate (PO_4 – 20 Mt of P) is mined (Fixen 2009, Van Kauwenberg 2010), of which approximately 90% is used as fertilizers, animal feed and food additives (Smil 2000). There are limited P reserves and it is expected to deplete in 50-120 years (IWMI 2007). There are concerns over global geological depositions of P, as most deposits are concentrated geographically in Saudi Arabia, Jordan, China, USA and Morocco (Cordell and White 2011, Prud'homme 2015). There is increased demand for P to sustain food production and feed an increasing global population. Due to limited recycling, a significant proportion of P from human and livestock waste streams makes its way towards water bodies (Filippelli 2002). This has caused serious environmental concerns such as algal bloom and eutrophication. Under these circumstances, recycling sufficient P from alternative resources will be a significant need in the future.

There are large amounts of P in waste streams originating from domestic and agricultural industries (Faostat 2015). Due to recent technology developments, almost 90% of PO_4 can be economically recovered from these waste streams (Mehta, Khunjar et al. 2015). The recovered P can be as struvite (magnesium ammonium phosphate), calcium phosphate, biosolids, ash from waste incineration and other P based fertilizers. Most of these products have been tested as agricultural additives, and most have commercial merit, either as soil additive or industrial P source (Suzuki, Tanaka et al. 2007, Ksawery, John et al. 2010, Sengupta and Pandit 2011, Antonini, Nguyen et al. 2012). There is an opportunity to recycle P from waste streams as a valuable agriculture product.

Anaerobic digestion (AD) is widely used to stabilize the waste streams and release P from organic fraction of the waste. Neutral pH conditions and the presence of cations in the waste streams provide favourable conditions for P-precipitation during AD. Some of these precipitants deposit and scale within the digester and in downstream accessories (Doyle, Oldring et al. 2002). Moreover, because it is precipitated, the P passes through to the biosolids during solids separation (Bradford-Hartke, Lane et al. 2015). This reduces the availability of P and reduces recovery yield for the P recovery technologies. Therefore, there is a need to investigate techniques to improve P solubility during AD process, which could potentially increase its recovery following AD.

1.2 Phosphorus in waste streams

P can be sourced from different waste streams produced by livestock (34%), chemical industry (7%), fertilizers (16%), municipal wastewater (34%), and others (9%) (Rahman, Salleh et al. 2014). In 2015, more than 2.1 billion ton (Bt) of livestock manure (dry) and 298 trillion litres (TL) of wastewater was generated from agricultural and municipal industries (Faostat 2015) (Table 1.1). P concentration in manure varies from 0.2 to 2.7% (Mehta 2016), while its concentration in the wastewater varies with the source, domestic wastewater effluent (< 10 mg L^{-1}) is considered as low strength, while piggery and meat processing wastewater (> 100 mg L^{-1}) are considered as high strength wastewater (Karunanithi, Szogi et al. 2015, Mehta 2016). The waste streams have a combined P potential of approximately 23.3 Mt every year, which can potentially reduce the global PO_4 rock production by 50% every year (Karunanithi, Szogi et al. 2015). In Australia, the total P potential from waste streams is around 92 kt per year which is around 40% of its agronomic demand, and most of the waste streams are located within 200 km of the grain producing regions of Australia (Mehta 2016). Hence, the recovered P from these waste streams could be cheaply transported and applied for agriculture application. There is similar potential from other agriculture and livestock intensive countries to recycle and reuse P from waste streams, and reduce consumption of the mined P (Karunanithi, Szogi et al. 2015).

Table 1.1: Total phosphorus concentrations in selected waste streams.

Source	Waste type	Solid waste streams (g-P kg^{-1})	liquid waste streams (mg-P L^{-1})
Sugarcane	Bagasse	0.7	-
	Molasses	1.0	-
	Mill mud	9.6	-
Beef feedlots	Feedlot	8.0	63
Dairy feedpads	Manure	6.0	26
Piggeries	Pond sludge	15	-
	Deep litter	11	-
Poultry (broiler)	Spent litter	18	-
Poultry (layers)	Caged bird manure	26	-
	Barn bird manure	10	-
	Fee range bird manure	10	-
Meat processing	Beef and sheep	23.6	47
	Pig processing	23.6	33
	Poultry processing	-	26
Milk processing	Waste	-	71
Fish processing	Solid waste	27	-
Municipal	Wastewater	27	2.7
Solid waste	Municipal	26	-
	Commercial and industrial	34	-
Electricity generation	Ash dams	0.6	-
	Ash from electricity generation	0.6	-

Note: Total phosphorus potential is calculated based on its average concentration in selected waste streams such as, effluents; cattle (4.5 g kg^{-1}), sheep (2.3 g kg^{-1}), piggery (7-9 g kg^{-1}), and municipal wastewater (14 mg L^{-1}). Source (Faostat 2015, Mehta 2016).

1.3 Forms of phosphorus in waste streams

P in waste streams can be broadly classified as inorganic and organic P. Organic P is contained in nucleic acids, phospholipids, inositol phosphates, phosphor amides, phosphor proteins, sugar phosphates, amino phosphoric acids and organic condensed P species (Majed, Li et al. 2012). Biodegradable organic P is hydrolysed into orthophosphate during the AD process, while non-biodegradable organic P will pass through a treatment facility and become the refractory portion in the final effluent (Lancaster and Madden 2008, Gu, Majed et al. 2009). Soluble inorganic P occurs as

orthophosphates (H_2PO_4, HPO_4^{2-}, PO_4^{3-}) and polyphosphates (Hammer and MacKichan 1981). P also binds with cations such as calcium (Ca), magnesium (Mg), aluminium (Al) and iron (Fe) to form insoluble inorganic P.

Table 1.2: Total phosphorus, inorganic phosphorus and phosphate concentration in wastewater used as feed for anaerobic digester.

Source	**T-P[4]**	**T-Pi[5]**	**T-PO_4[6]**	**Reference**
Black water[1]	220	79	141	(De Graaff, Temmink et al. 2010)
Municipal WW[1]	3.5	2.2	1.3	(Kim and Nakhla 2009)
Activated sludge	96	12	84	(De Haas, Wentzel et al. 2000)
	89	16	73	
	7	4.8	2	
Waste Activated Sludge	104	13	44	
(EBPR)[2]	186	16	79	
	241	13	116	
Waste Activated Sludge[3]	470	150	320	(Bi, Guo et al. 2012)
Waste Activated Sludge [1]	350	190	160	(Stratful, Scrimshaw et al. 2001)
	441	6	435	(Lee and Han 2013)
	155	151	4.1	(Pastor, Mangin et al. 2010)
Dairy stockpile manure as solid	11000	7810	3190	(Hansen, Cade-Menun et al. 2004)
Dairy lagoon manure as liquid	120	37	14	
Dairy manure	12	-	-	(Güngör, Jürgensen et al. 2007)
Dairy manure[3]	890	700	190	(Güngör and Karthikeyan 2008)
Pig slurry[3]	493	424	69	(Ndegwa 2004)
Pig manure[3]	710	646	64	(Luo, Zhu et al. 2002)

[1]T-Pi as PO_4
[2]With acetate supplement
[3]T-Pi as dissolved reactive P
[4]Total phosphorus (mg L^{-1})
[5]Total inorganic phosphorus (mg L^{-1})
[6]Total organic phosphorus (mg L^{-1})

The fraction of organic and inorganic P varies with waste stream type and also changes during the treatment train, from source to final disposal. On an average, 60-90% of P in manures is present as inorganic P, while the remainder is contained in manure as organic P (Barnett 1994). Meat processing wastewater and poultry manure contain 50% of the total P as inorganic P, while dairy and swine manure slurry carry 70 and 86% as inorganic P. Domestic wastewater, originating from toilets, contains organic and inorganic P as 64 and 36% of total P (220 mg L^{-1}) respectively (Table 1.2). The concentration of P changes when the wastewater is mixed with other household sources, making a total P concentration of 3.5 mg L^{-1}, and the organic P content is diluted from 37 to 64% (Kim and

Nakhla 2009). This highly diluted stream is concentrated (in P) through waste activated sludge (WAS), clarification, and post-thickening, making waste activated sludge (WAS) as a final feed for the digester, with a total P concentration of 350-470 mg L^{-1} achieved, having 60-90% as organic P (Stratful, Scrimshaw et al. 2001). Nearly 100% of P is converted to inorganic P at the completion of AD process, with 90% of P in the form of insoluble inorganics (Bradford-Hartke, Lane et al. 2015).

1.4 Fate of phosphorus during anaerobic digestion

Anaerobic bioconversion of organic waste is an established method to minimise environmental impact, and can be applied to an extensive range of waste streams; municipal, industrial, and agricultural effluents and wastes (Angelidaki, Ellegaard et al. 2003). This process includes four major steps; hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Fig. 1.1. In the first stage of AD process, complex organic matter such as carbohydrates, proteins, and fats are converted into sugars, amino acids and long chain fatty acids. The organic matter further disintergrates into soluble substrates that are utilized by microorganisms. The microorganisms that mainly consist of bacteria are the obligate and facultative anaerobes, responsible for the hydrolysis process, and remove a small portion of oxygen from the reactor sludge (Parawira, Murto et al. 2004). In the second step, simple organic substrates are converted into short chain fatty acids by microbes where they produce acetic, propionic, butyric, valeric, hexanonic acids, and etc. The acidogen-methanogen balance is important because the acids generated may not be consumed directly by the methanogens, because the acidogenic community has a faster growth rate than methanogens (Mosey 1983). The acidogenic bacteria are the main driver of biochemical reactions linked to this phase, but are sensitive to the operational conditions such as pH and temperature that may influence the concentration of acids in the reactor sludge (Ho 2014). In the final stage, acetogens convert short chain organic acids, to acetate, hydrogen (H_2) and carbon dioxide (CO_2). In the final stage, methanogenesis takes place, which produces methane (CH_4) and CO_2 as a by-product.

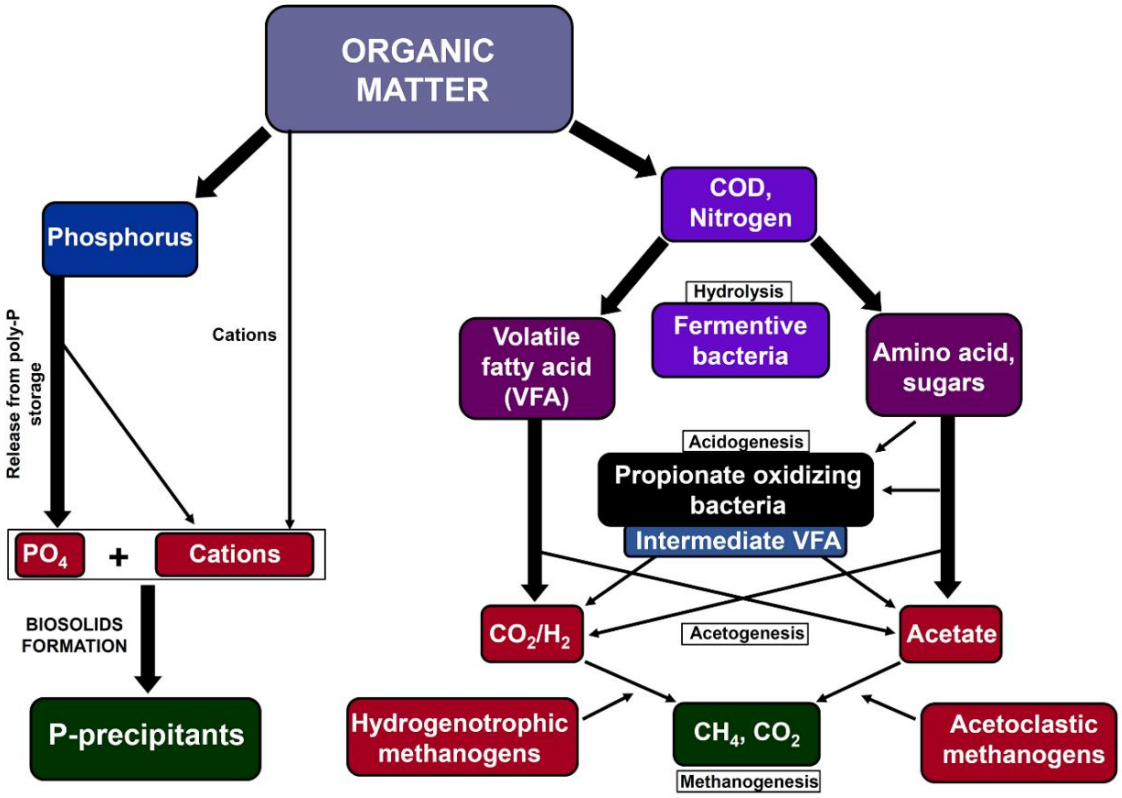

Figure 1.1: A typical sludge decomposition pathway along with phosphorus precipitation during anaerobic digestion process at neutral pH conditions (Gerardi 2006).

The four key stages of the AD process demonstrate the disintegration of organic matter into energy producing products, parallel to these, key nutrients (Ca, Mg, P, N and Fe) are released, and forms are changed within the anaerobic digester (Fig. 1.1). During AD, nutrient concentrations are conserved and they do not take part in the AD process except small amounts used for cell synthesis (Ghasimi, Idris et al. 2009). The P is released as inorganic PO_4 from organic P during AD (Gerardi 2006). The released PO_4 tends to precipitate with cations such as Fe, Al, Mg and Ca to form an insoluble complexes such as $FePO_4$, $AlPO_4$, $MgNH_4PO_4$ (struvite) and $CaPO_4$. Some of these precipitants deposit and scale within the digester and in downstream unit operations (Doyle, Oldring et al. 2002). These depositions can disrupt operation and result in significant expenses. The P precipitant cleaning cost is estimated to be between A$2000 to A$10000 per year depending upon the capacity of a WWTP (Shu, Schneider et al. 2006). Most of these precipitants are separated in the biosolids, with almost 90% of P inlet during the waste treatment ends up in the biosolids (Banister, Pitman et al. 1998). The disposal or treatment of P-rich biosolids can be expensive, due to limited application and regulations on application of biosolids on agricultural land. There are risks associated with the availability of POPs (persistent organic pollutants) and emerging pollutants (such as pharmacutecal and personal care prodcuts) in biosolids (Clarke and Cummins 2015). In addition, the

P binds with Al and Fe to form complexes, and such complexes have limited plant availability due to low solubility and depressed plant uptake (Sarkar and O'Connor 2004, Brännvall, Nilsson et al. 2014).

An alternative approach is required to reduce unwanted P precipiation and reduce P fraction in biosolids, and increase PO_4 concentration in the digestate following AD. Several techniques have been developed with commercial application to recover more than 90% of soluble inorganic P from filtered digestate (Mehta, Khunjar et al. 2015) as controlled release inorganic fertilizer. Recycling P through such technologies is emerging not only for sustainability reasons but also due to economic drivers based around the supply-demand issues outlined above in section 1.1.

It is important to clarify that inorganic P is released from organically bound P during AD due to the decay of cellular biomass, as well as hydrolysis of poly-P. This released and soluble P will tend to precipitate with NH_4^+, Mg^{2+}, Ca^{2+}, Al^{3+}, and Fe^{3+}, commonly found ions in digestate. This reduces availability of soluble P following anaerobic digestion. Therefore, enabling increased soluble P concentration in AD is the main focus of this thesis, with various techniques to enhance soluble P levels, as highlighted in the next section.

1.5 Techniques to increase phosphorus solubility during anaerobic digestion

As shown in the previous section, AD is a complex process that involves a series of chemical and biological reactions to break down organic material in the absence of oxygen, and to produce methane. There is a lack of information on the enhancement of soluble P concentration during AD. However, there are several operational parameters which could escalate soluble P levels in the digested sludge, can reduce P precipitation in an anaerobic digester such as temperature, pH and pressure. These parameters are known to influence biodegradability and methane production if varied substantially. However, none of these parameters have been investigated to improve soluble P concentration during digestion, and it is difficult to determine their influence on P forms and concentrations hypothetically. Table 1.3 highlights potential use of these parameters, showing their influence on methane production, PO_4 solubility, cation and heavy metals removal, and overall benefits and weaknesses.

Table 1.3: Summary of possible techniques to enhance phosphate solubility during anaerobic digestion.

Technique	Fundamentals	Type	Influence on AD	Enhancement of soluble P conc.	Substrate Treated	Benefits	Weakness	References
pH	Hydrogenation / acidification	Acid	CH_4 production reduced due to the accumulation of VFA	P released as soluble P up to 80% of total P concentration at pH 5.0	WAS	High PO_4 and cations	High cost, loss of CH_4, needs further justifications	[1 – 2]
Temperature	Hydrolyses	Thermophilic	CH_4 improved at thermophilic temperatures	Influence is negligible	Various waste streams	Higher CH_4, reduced HRT	PO_4 and cations unaffected, need further justifications.	[3]
		Mesophilic	n.a.	P precipitation occurred	Primary sludge	Improved P and N solubility		[4]
Pressure	Sludge integration/CO_2 solubility	Self-generative	Improved CH_4 contents in biogas followed by higher biogas production	Not reported	Various substrates	Biogas upgradation	PO_4 release not studied, need further justifications in single stage AD.	[5-8]
				Not reported	Algae	Potential to solubilize P and N		[9]

Note: [1] (Chen, Jiang et al. 2007), [2] (Bi, Guo et al. 2012), [3] (Ho 2014), [4] (Banister, Pitman et al. 1998), [5] (Lindeboom, Fermoso et al. 2011), [6] (Lindeboom, Weijma et al. 2012), [7] (Zhang, Zhang et al. 2012), [8] (Fang, Zhang et al. 2014), [9] (Keymer, Ruffell et al. 2013).

1.5.1 Temperature

Temperature can influence AD performance through controlling and restraining microbial community composition and diversity, and thermodynamic equilibrium of the biochemical reactions (Wilson, Murthy et al. 2008). For example, methane production increases as temperature shifts from 37 to 55 °C, which is due to an increase in hydrogenotrophic methanogens and decrease in acetoclastic methanogens (Pap, Györkei et al. 2015). High temperature conditions can also enhance solubility of key precipitants during AD, and could increase PO_4 concentration during thermophilic AD (55 °C). The solubility of struvite, a key P precipitant during AD, at various temperatures is shown in Fig. 1.2. The struvite solubility increased with temperature from 0 to 20 °C (Boswell, Dick et al. 1999), followed by drop in solubility above 35 °C. A study showed that the Ca-P complexes were marginally influenced by temperature in the range of 5 – 30 °C (5 – 10% increase), but increased linearly with increasing P concentrations and temperature (Song, Hahn et al. 2002). Temperature can influence the solubility of the P-complexes up to 30 °C, but the effect of mesophilic and thermophilic temperatures on PO_4 concentration is ineffective (Bolzonella, Cavinato et al. 2012). Overall, temperature has a very limited impact on any of the P complexes, and hence, even more limited impact on the overall PO_4 concentration.

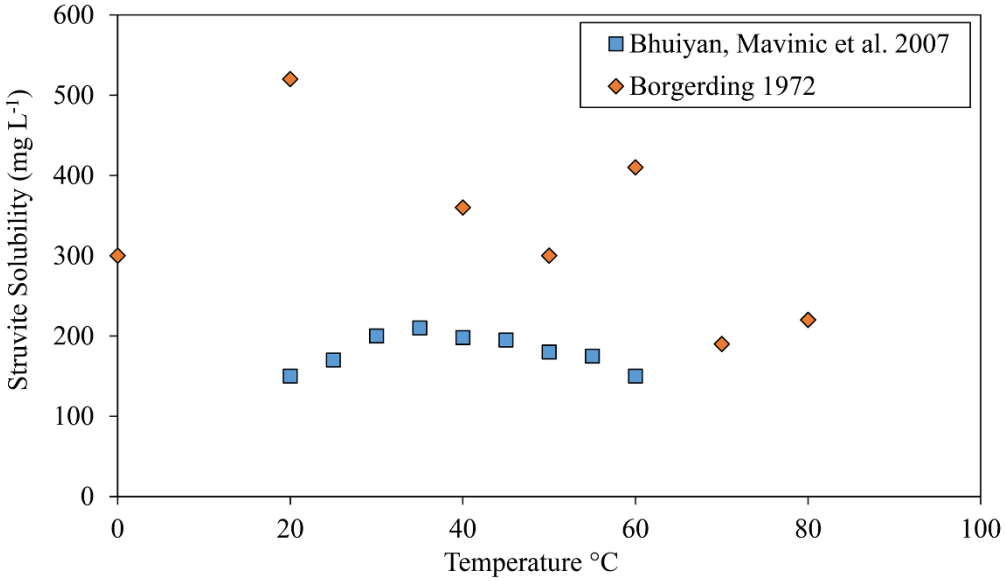

Figure 1.2: Solubility of struvite in the temperature range 20 – 80°C.

1.5.2 pH

An acidic pH moves the chemical equilibrium and increases availability of phosphate ions ($HPO_4^{2-} \rightarrow PO_4^{3-}$), which generally increase solubility of P complexes (since P is limiting ion for

precipitation), while alkaline pH results in P-precipitation as Mg-P or Ca-P based compounds (Sommer and Husted 1995, Nelson, Mikkelsen et al. 2003, Burton 2007, Christensen, Hjorth et al. 2009). Struvite solubility is lowest between pH 8 and 10, as shown in the Fig. 1.3. This reveals that for the practical range of AD pH values (< 9.0), solubility is always enhanced with a decrease in pH. AD operation at depressed pH have been recommended to increase P solubility (Banister, Pitman et al. 1998, Chen, Jiang et al. 2007, Wu, Yang et al. 2009, Bi, Guo et al. 2012) and to avoid any deposition-precipitation in the digester. However, low pH conditions can impact on methane production (Beccari, Bonemazzi et al. 1996, Gerardi 2006) by accumulating VFA. Based on the literature discussed, a combined overview reveals that a low pH AD operation has potential to enhace PO_4 levels in the reactor, with the provison that methane productivity may be impacted.

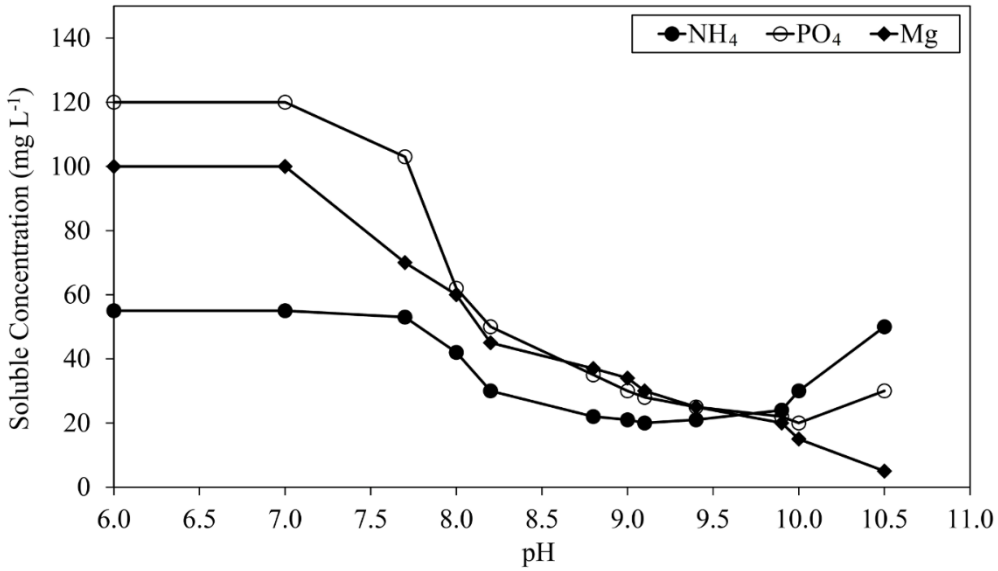

Figure 1.3: Concentration of ammonium, phosphate and magnesium at various pH conditions. Source (Booker, Priestley et al. 1999).

1.5.3 High pressure

Increased pressure increases CO_2 solubility in a solution, which is in equilibrium with the weak acid as carbonic acid (H_2CO_3). The formation of carbonc acid causes a slight decrease in pH of the solution (Meyssami 1992). The formation of carbionic acid can be written as a chemcial reaction (Eq. 1.1) (Morel and Hering 1993).

$$\mathbf{CO_{2(g)} + H_2O_{(aq)} \leftrightarrow H_2CO_{3(aq)}} \qquad \text{(Eq. 1.1)}$$

The reaction in equation 1.1 is in equilibrium, which means the pH of digester contents is immediately impacted by increased carbonic acid concentrations. AD is typically operated at ambient pressure, which results in CO_2 content in the biogas are 30 – 40% of biogas, which can be reduced if the digester is operated at higher pressure. Since CO_2 is 20 times more soluble than CH_4, with Henry's constants for CO_2 and CH_4 of 0.031 and 0.0016 mol L^{-1} bar^{-1} at 30 °C, respectively (Wang, Chen et al. 2003). This means that CO_2 will preferentially dissolve into liquid at elevated pressures, and that increased pressure will result in a higher biogas methane composition. Several studies have been conducted at high pressure conditions, which were mostly focussed on either sludge hydrolysis or biogas upgrading (Bamberger, Sieder et al. 2000, Rai and Rao 2009, Lindeboom, Fermoso et al. 2011, Keymer, Ruffell et al. 2013, Zhang, Zhang et al. 2013, Chen, Rößler et al. 2014, Wahidunnabi and Eskicioglu 2014). Some of these studies found methane content up to 95% of biogas at a pressure range of 2 – 90 bar, this also resulted in a two fold increase in methane production. In theory, the increase in CO_2 in the liquid should enable a reduced pH, which will increase PO_4 concentration, and possibly also due to an increase in ionic strength, but no results regarding enhancement in PO_4 concentration have been published.

1.6 Research objectives and approach

The literature suggests that pH and pressure could increase PO_4 concentration during AD, but these parameters need further justificaton. A low pH could increase PO_4 concentration during AD, but methane produciton is compromised. However, there is still a lack of informaton on low pH AD in the literature. A high pressure is reported to be effective to enhance methane contents in biogas by solubilizing CO_2 in the reactor sludge, but this technique is also not investigated in a perspective of increasing PO_4 concentration, especially in a single stage AD process. Based on the limited literature available on the influence of low pH and high pressure on PO_4 during AD, three specific research goals have been formulated of which first two are typically based on low pH AD. This research addressed engineering, physico-chemical and microbial aspects of the AD of WAS using low pH and high pressure techniques. A series of laboratory scale experiments have been conducted in both batch and single stage continuous AD to improve PO_4 concentration. The research objective and approach for this thesis is as follows.

1. **To investigate the effect of low pH on PO_4 concentration during batch anaerobic digestion of waste activated sludge**

This study investigates the effect of low pH on PO_4 concentration during mesophilic batch AD, which will set a bench mark of pH optimization. Four low pH conditions (pH 6.5, 6.0, 5.5 and 5.0) will be compared with the control (pH 7.0) using biochemical methane potential (BMP) testing. Two sets of BMP experiments will be carried out, with target pH levels of 5, 5.7 and 6.5 in the first batch and, pH 5, 5.7, 7, and 7.2 (no adjustment control) in a second batch. Blank reactors will be set up in both batches to account for native inoculum production. Briefly, excess serum flasks will be set up and sacrificially titrated during adjustment events to determine the amount needed to adjust the remaining flasks using 2 M HCl (with triplicates remaining at the end of the batches). Batches 1 and 2 will first be adjusted at 7 d and 1.5 d respectively. Further pH adjusted will be carried out depending on the maximum methane contents at each pH, and these will be at 15, 29, 36, and 51 for batch 1, and 9, 21, 35 and 51 days for batch 2. Each BMP experiment will be conducted for 51 days. Elemental concentration along with methane production, VFAs and soluble chemical oxygen demand (SCOD) are measured at each pH adjustment interval, while microbial community analysis (pyrotag sequencing) will be done at day 35 of both batch tests. Further experimental details are provided in Chapter 2.

2. **To investigate the effect of low pH on PO_4 concentration during continuous anaerobic digestion of waste activated sludge**

The second objective will be developed based on the outcomes of batch experiments. In this study, a continuous low pH AD will be conducted. Continuous anaerobic reactors will operate using a 12-day HRT and an organic loading rate (OLR) of 1.91 ± 0.04 gCOD L^{-1} d^{-1}. Since anaerobic digesters normally run at relatively longer retention time (ranging 20-40 days) to produce maximum amount of methane from biomass, a 12-day HRT will be selected for moderate operation, which is suitable to identify a kinetic response to the treatment, also based on BMP results. The OLR will be pre-determined based on HRT and working volume of 1L for continuous experiments. Three identical anaerobic reactors (one control and two test) will be operated for a total period of 202 days. Test reactor 1 will operate at 7.0, 6.5, 6.0 and 5.5, at a minimum of four HRTs for each pH set point. A test reactor 2 will be added later in the experiment, and it will operate at pH 7.0 for the first five weeks followed by pH 5.0 for the next four HRTs. The pH will be adjusted using 1 M HCl solution. The operation time at each pH set point will be based on the convergence of both test and control reactors prior to application of new pH conditions (that is between 35-48 days – when data will become stable

and no major variation will be observed). Total solids, volatile solids, COD and biogas contents will be measured three times a week, VFA and SCOD twice a week, soluble elements once a week, and microbial community prior to shifting to a new pH. Further experimental details are provided in Chapter 3.

3. To investigate the effect of high pressure on PO_4 concentration during continuous anaerobic digestion of waste activated sludge

This objective will be set to avoid acid addition during AD. The purpose of this technique is to depress the reactor pH by solubilizing headspace CO_2 using high pressure AD (HiPAD) technique. In this study, CO_2 will be solubilized to reduce pH by running the anaerobic reactor at 1.0, 2.0, 4.0 and 6.0 bars absolute (a) pressure along with a control at 1.0 bar (a), with a 12-day HRT, OLR of 1.91 ± 0.03 gCOD L^{-1} d^{-1}, and a flow rate of 63 ± 2 mL d^{-1}, maintaining a working volume of 750 mL. A stainless steel anaerobic reactor will be designed and fabricated locally using pressure safety standards, and will be verified for its application from authorized pressure testing agency. Total solids, volatile solids, COD and biogas contents will be measured three times a week, VFA and SCOD twice a week, soluble elements once a week, and microbial community before applying new pressure set point. This experiment is planned to run for a minimum 180 days, providing up to four HRTs at each pressure. Further experimental details are provided in Chapter 4.

2. LOW pH ANAEROBIC DIGESTION OF WASTE ACTIVATED SLUDGE FOR ENHANCED PHOSPHOROUS RELEASE

2.1 Introduction

Phosphorus (P) solubilization during anaerobic digestion (AD) is vital to enhance its recovery. As soluble fraction of P always tries to bind with available cation such as calcium (Ca) and magnesium (Mg), a lesser amount of P remained in the sludge supernatant due to its unwanted precipitation in the reactor and biosolids. It has been widely reported that almost 90% of the total P ends up in sludge biosolids, while rest of it goes to the sludge supernatant (Smil 2000, Bradford-Hartke, Lane et al. 2015). About all of the supernatant P is recoverable as struvite, but a minor fraction is recoverable from biosolids due to P binding with iron (Fe) and aluminum (Al). The Fe-P and Al-P is inaccessible by plants thus most of the P rests in soil column and finally flushed out with run off. Therefore, in-reactor P solubilization is important to maximize P recovery post AD. The in-reactor P solubility can be increased by low pH AD. Low pH conditions (pH 5.0 – 5.5) increase hydrogen ion concentration in the sludge liquor that allows the precipitates to dissociate, thus liberating more phosphate (PO_4) into the solution. On the other hand, low pH causes a reduction in methane production due to the accumulation of volatile fatty acids (VFAs) that could cease whole AD process. Therefore, this chapter address a preliminary study on low pH AD options to enhance P solubility.

Redrafted after

Muhammad A. Latif, Chirag M. Mehta, Damien J. Batstone. (2015). "Low pH anaerobic digestion of waste activated sludge for enhanced phosphorous release." Water Research 81: 288-293.

2.2 Materials and methods

Two sets of batch experiments were done, with target pH levels of 5, 5.7 and 6.5 in the first batch and, pH 5, 5.7, 7, and 7.2 (no adjustment control) in a second batch. Samples for batches 1 and 2 were collected in winter and summer respectively. There was no motivation to collect batches in different seasons except the sampling time which was due for that batch. Blank reactors were set up in both batches to account for native inoculum production. Briefly, excess serum flasks were set up and sacrificially titrated during adjustment events to determine the amount needed to adjust the remaining flasks using 2M HCl (with triplicates remaining at the end of the batches). Batches 1 and 2 were first

adjusted at 7d and 1.5d respectively. Microbial community analysis (pyrotag sequencing) was done at 35d in both batch tests.

2.2.1 Substrate and inoculum

The substrate (WAS; waste activated sludge) and inoculum (ADS; anaerobic digested sludge) were collected from a sewage treatment plant operated by Queensland Urban Utilities, Brisbane. The substrate was collected from dissolved air floatation (DAF) unit at 3% total solids and was stored at 4 °C. It was further thickened by removing excessive water after 24 hours of storage. The inoculum was collected from mesophilic anaerobic digester treating a mixture of primary and activated sludges. It was degassed i.e. pre-incubated at 37 °C for one week in order to deplete the residual biodegradable organic material. The physico-chemical properties of substrates and inoculums are shown in Table 2.1. The substrate was a grab sample for each batch experiment with the serum flask agitated manually prior to sampling to ensure a representative sample.

Table 2.1: Physico-chemical properties of substrate and inoculum of batch 1 and 2.

Parameter	**Substrate**		**Inoculum**		**Units**
	Batch 1	**Batch 2**	**Batch 1**	**Batch 2**	
pH	6.4	6.7	7.2	7.2	-
COD	44	56	26	26	(g L^{-1})
TS	44	445	30	26	(g L^{-1})
VS	33	34	21	16.5	(g L^{-1})
Total P	1060	922	568	476	(mg L^{-1})
Soluble Ca	78	72	28	32	(mg L^{-1})
Soluble Fe	21	20	1	0	(mg L^{-1})
Soluble Mg	143	121	13	22	(mg L^{-1})
Soluble Na	373	281	406	398	(mg L^{-1})
Soluble K	215	207	348	240	(mg L^{-1})
TKN	1700	3420	2310	2720	(mg L^{-1})
NH_4-N	34	49	1324	1140	(mg L^{-1})
NOx-N	4	0	2	1	(mg L^{-1})
PO_4-P	321	340	385	398	(mg L^{-1})
VFA	7	33	5	12	(mg L^{-1})
TOC	118	125	168	227	(mg L^{-1})
TIC	86	95	990	939	(mg L^{-1})

2.2.1.1 Sewage wastewater treatment plant

The substrate and inoculum were sourced from Luggage Point Wastewater Treatment Plant (Plate 2.1). This is a major wastewater treatment plant in Brisbane, Australia, which treats a mix of domestic and industrial wastewaters, with design load of 750,000 equivalent persons. Over the experimental period (2014-2015), the average influent flow rate of the treatment plant was about 144 ML d^{-1}. The treatment plant includes conventional primary treatment units in parallel, the sludge from the primary treatment is fed to the two rotary screen thickeners. The effluent from the thickeners is sent to a number of parallel bio-reactors (two anaerobic, two anaerobic/anoxic, four anoxic, two aerobic/anoxic, three aerobic, one de-aeration, two post anoxic, and one re-aeration unit). The sludge is pumped from the last bioreactor (at a flow rate of 5600 m^3 d^{-1}) to a clarifier. A fraction of settled solids from the clarifier are transported to the bioreactors as returned activated sludge and rest (WAS) is thickened in four dissolved air floatation (DAF) unit. The thickened primary sludge and WAS is fed to one of the six anaerobic digesters, with a design HRT of 20 days and mesophilic temperature (35 – 37 °C). Each anaerobic digester has a working volume of 5000 m^3, with a gas lance mixing system and liquid recirculation. The biogas is collected from the digester's floating headspace (about 700 m^3) and used for cogeneration. Digested sludge is mechanically dewatered in centrifuges to up to 20% dry solids prior to offsite transport.

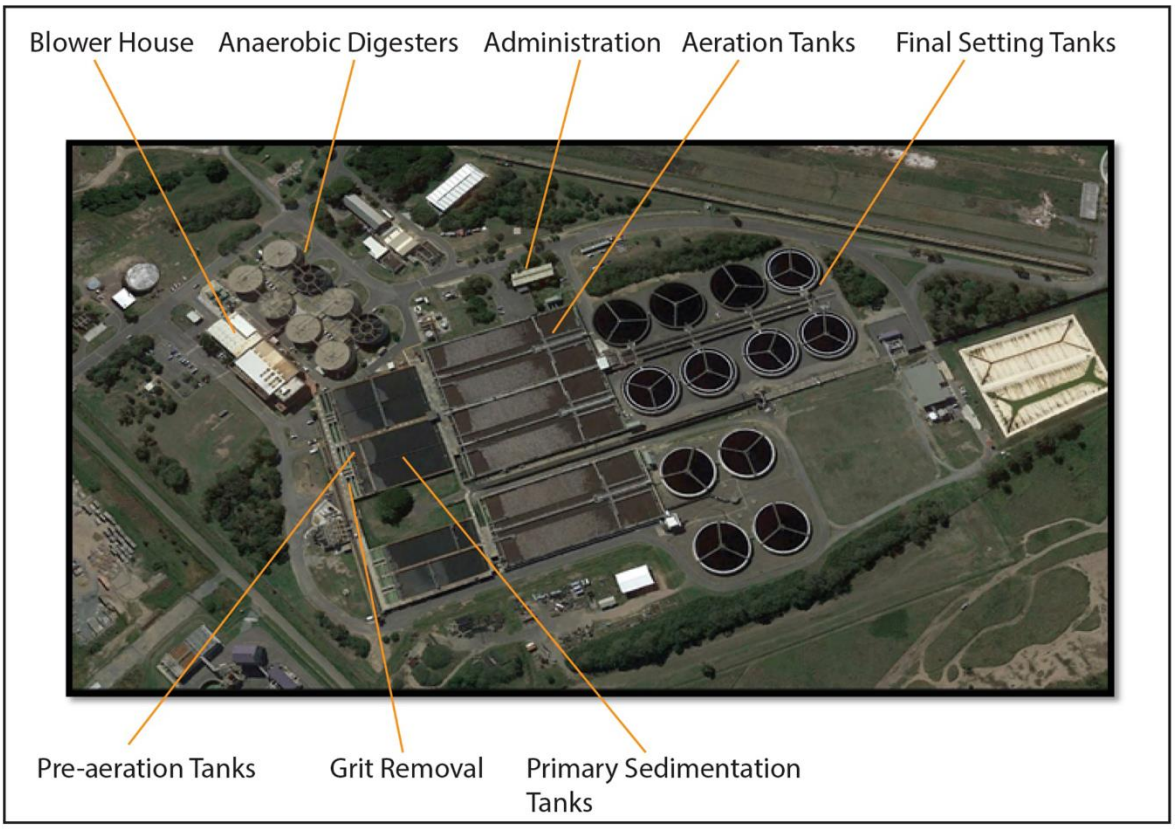

Plate 2-1: Luggage point wastewater treatment plant.

2.2.2 Biochemical Methane Potential (BMP) test

A modified biochemical methane potential (BMP) test (Jensen, Ge et al. 2011) was used for both batch tests (Fig. 2.1). The BMP tests were conducted in 160 mL glass serum flasks with a working volume of 100 mL. For batch 1, 28 mL of substrate and 72 mL of inoculum while 23 mL of substrate and 77 mL of inoculum were used in batch 2 to achieve inoculum to substrate ration (ISR) of 1.62 (VS basis). An ISR ratio of 1-2 is consistent with normal practice (Jensen, Ge et al. 2011), and while for poorly degradable substrates such as WAS, it results in a substantial methane contribution from the blank, error around this is relatively low. Each reactor was filled with 100 mL of assay and purged with N_2 gas at a flow rate of 4-5 L min^{-1} for 30 seconds. Each reactor was immediately closed with butyl rubber after N_2 purge, sealed with an aluminum crimp and placed in an incubator at 37 ± 1 °C to provide anaerobic conditions. Further experimental details are given in Angelidaki, Alves et al. (2009).

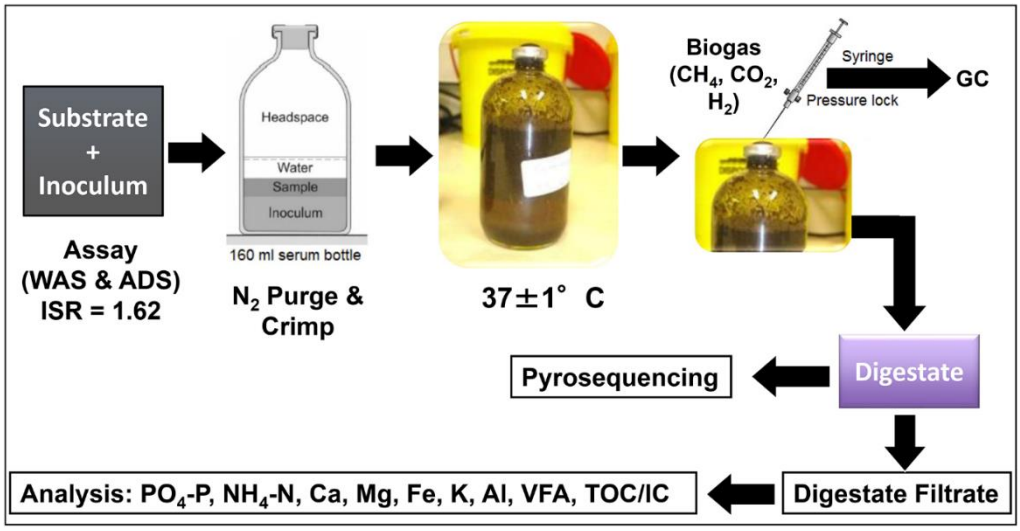

Figure 2.1: Biochemical methane potential test setup for 51 days batch time.

Two samples of WAS were analyzed in two large experiments as briefly described at the start of section 2. Initially, the reactors were incubated at pH 6.5 using 2M HCl in batches 1 and 2 for 7d and 1.5d respectively to establish activity. After initial incubation periods, one of the reactors was sacrificed (opened) for analysis and to estimate the amount of acid required to achieve targeted pH in other reactors by titration. The pH of the remaining reactors was then adjusted using this amount of acid, assuming buffering of the sacrificed reactor is similar to the remaining reactors (Fig. 2.2). Following this, at each pH condition, one reactor was sacrificed (4 per adjustment event). The adjustment interval and amount of acid added in each reactor can be found in Table 2.2 and was based on expected methane profile. Triplicate reactors at each pH condition remained at the end of the batch.

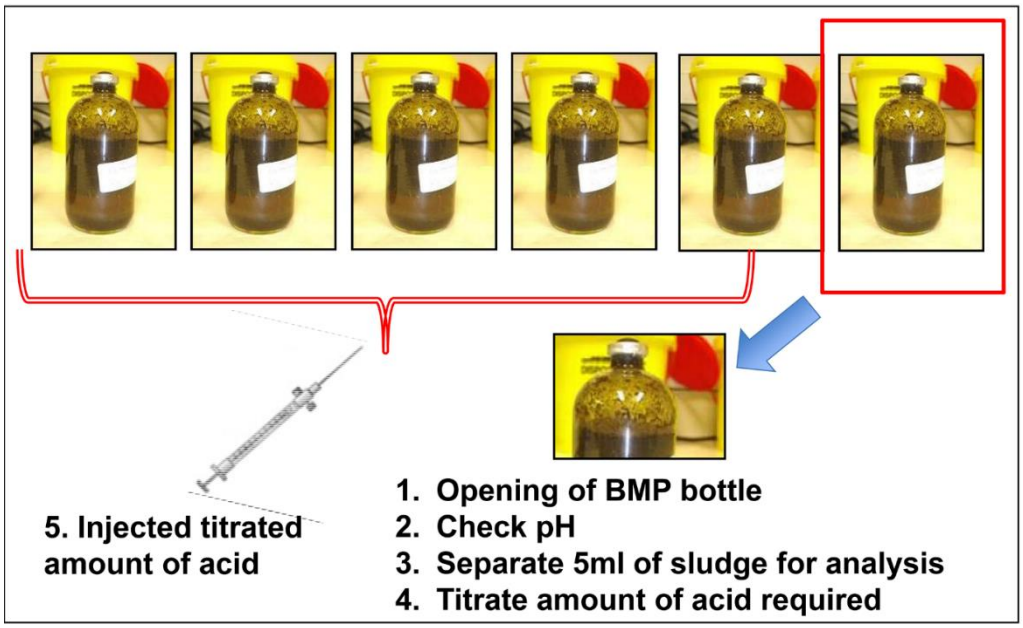

Figure 2.2: Sacrificing method of modified biochemical methane potential test.

Table 2.2: Sacrificing schedule and amount of acid used to achieve desired pH in both experiments

Batch 1				**Batch 2**			
Days	**pH[1]**	**pH[2]**	**HCl (ml)**	**Days**	**pH[1]**	**pH[2]**	**HCl[3] (ml)**
7	6.8	5.0	7.6	**1.5**	6.8	5.0	3.2
15	6.0	5.0	2.5	**9**	6.0	5.0	0.8
29	5.7	5.0	0.6	**21**	5.6	5.0	0.4
36	5.3	5.0	0.2	**35**	5.8	5.0	0.6
51	5.3			**51**	5.6		
7	6.8	5.7	6.1	**1.5**	6.8	5.7	2.4
15	6.2	5.7	1.6	**9**	6.3	5.7	0.6
29	6.2	5.7	0.9	**21**	6.1	5.7	0.5
36	6.0	5.7	0.3	**35**	6.0	5.7	0.3
51	6.2			**51**	6.4		
7	6.8	6.5	2.3	**1.5**	6.8	7.0	0.2[4]
15	6.5	6.5	0.4	**9**	7.0	7.0	0.0
29	6.8	6.5	0.9	**21**	7.0	7.0	0.0
36	6.9	6.5	0.3	**35**	7.1	7.0	0.0
51	6.9			**51**	7.6		

[1] Recorded pH
[2] Adjusted pH
[3] as 2M HCl
[4] as 1M NaOH

The methane yield from sample free blanks was subtracted from the batch-containing sample (using the model as in Eq. 2.1) and blank corrected data was fitted with the relationship:

$$B = B_0 \cdot (1 - e^{-k_{hyd}t}) \quad \text{(Eq. 2.1)}$$

Where B is the methane production in $mLCH_4\ gVS_{fed}^{-1}$, B_0 is the biochemical methane potential in $mLCH_4\ gVS_{fed}^{-1}$ at 25 °C, and 1 bar, k_{hyd} is the first-order degradation rate coefficient (d^{-1}) and t is the batch time (days). The lsqcurvefit() function in MATLAB was used to estimate parameter values in Eq. 2.1, together with parameter errors calculated from linear estimate of errors based on a two-tailed t-test (95% confidence interval). Only data following the first pH adjustment was used for parameter estimation.

2.2.3 Physico-chemical analysis

Chemical oxygen demand (COD) was measured according to Standard Methods (1980). Lachat Instruments USA, Quick Chem 8000 flow injection analyzer (FIA) was used to measure PO_4^{3-}-P and NH_4^+-N. PerkinElmer, USA Optima 7300 DV inductivity coupled plasma-optical emission spectroscopy (ICP-OES) equipped with WinLab32 for ICP software was used to measure soluble and total metal ions along with the total Kjeldahl phosphorus (TKP) and nitrogen (TKN). Gas chromatograph (Agilent model 78090A, USA) with flame ionization detection was used to measure volatile fatty acids (VFAs). Total organic and inorganic carbon were analyzed by using Analytik Jena, Germany (model Multi N/C 2100 S). Biogas production was recorded at regular intervals by measuring the biogas pressure in serum reactors. Pressure measurements were obtained using a water-filled manometer (pH acidified). Biogas volumes were converted to an equivalent volume at standard room temperature and pressure (25 ºC and 1 atm) using the ideal gas law. Gas composition (CH_4 and CO_2) was measured using gas chromatography (GC). The GC used was a Perkin Elmer, USA auto system GC equipped with a thermal conductivity detector and a 2.44 m stainless steel column packed with Hayesep Q (80/100 mesh). The GC was calibrated using external gas standards obtained from British Oxygen Company (BOC).

2.2.4 Community profiling

The analysis of microbial community structure and diversity was examined using pyro sequencing from samples of both batches taken at day 35 assuming that these have well stabled at applied pH conditions. Total DNA was extracted from sludge samples using FastDNA® Spin Kit for soil (MP Biomedicals, California, USA). Samples were prepared according to the protocol provided by MP Biomedicals for DNA extraction. The primers (single-stranded DNA molecules) used for pyrosequencing were universal primers 962f (5'-AAACTYAA AKGAATTGACGG-3') and 1392r

(5'-ACGGGCGGTGTGTAC-3'). Sequencing was carried out using a Roche 454 GS FLX sequencer (Roche, Switzerland). The purity of the extracted DNA was checked by calculating A_{260}/A_{280} ratios using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technology, Rockland, DE). In addition, the products were examined by agarose gel (1%, w/v) electrophoresis performed at 70 V for 40 min. The gel was stained with ethidium bromide and photographed under UV light with a Gel Doc™ XR camera (Molecular Imager, Bio-Rad, USA). The isolated DNA was then stored at -20 °C until further use.

Molecular Evolutionary Genetics Analysis (MEGA) version 5.2 software was used for maximum likelihood analysis of bacteria/archaea to select best-fit substitutions of nucleotides (Tamura, Peterson et al. 2011). Influence of batch and pH was done through ANCOVA as described above, with pH as a regressor, and batch number as a categorical factor. Actual pH when sampled (day 35) was used as regressor, which was different only for the highest two experiments from batch 2 (7.05 and 7.17 at day 35 vs 7.6 and 7.7 at day 51).

2.2.5 Other statistical analysis

To test the effect of pH and batch set on B_0 and k_{hyd}, analysis of correlated variance (ANCOVA) was done using the MATLAB function anovan(), with batch number (1 or 2) as a categorical variable, and pH as a regressor (continuous variable). Standard linear regression has been used elsewhere for correlation analysis. p-values are provided for significance testing, with a conventional 5% threshold ($p<0.05$) applied to identify a weak statistically significant relationship. Confidence intervals (CI) provided on graphs and in tables are likewise based on a two-tailed *t*-test with a significance threshold of 5% (95% CI) in the mean of replicate analyses.

Error in blank methane potential was 2.9 $mLCH_4\ gVS_{fed}^{-1}$ for batch 1, and 3.6 $mLCH_4\ gVS_{fed}^{-1}$ (95% CI) for batch 2, and hence as a small and static error (not varying within experiment sets or across replicates), its contribution was not propagated through Eq. 2.1 for error in B_0. The effect of inoculum (and hence uncertainty in inoculum potential) was accounted for implicitly in the ANOVA as outlined above.

2.3 Results and Discussion

2.3.1 Effect of pH on biochemical methane potential

The methane yield curves fit well to the first order model with minimal scatter between triplicates (Fig. 2.3). Waste activated sludge degradability (B_0) was 214 ± 12 $mLCH_4$ gVS_{fed}^{-1} and first order coefficient (k_{hyd}) was 0.15 ± 0.03 d^{-1} at neutral conditions which is similar to previously reported (Wang, Jiang et al. 2014).

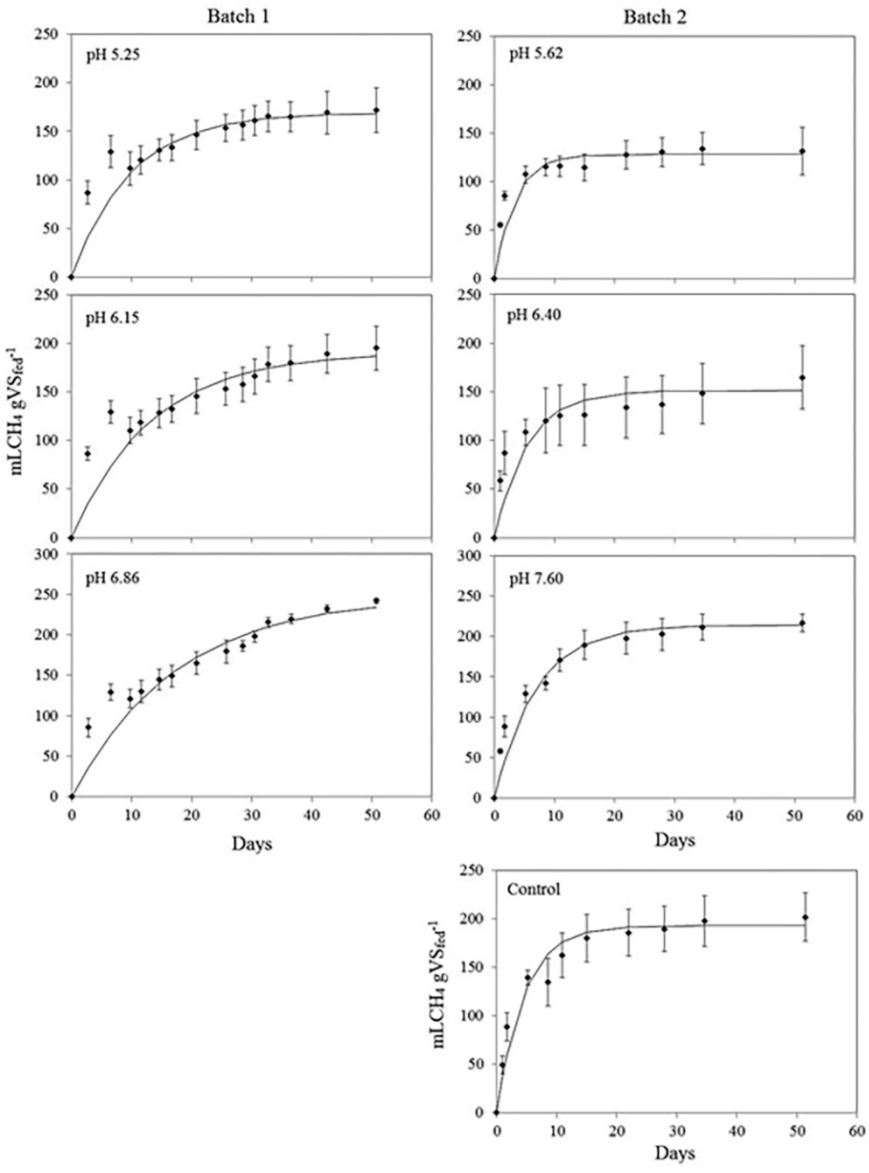

Figure 2.3: Methane production per gVS_{fed} at different pH conditions during batch 1 and 2. The data points are the actual methane produced per gVS_{fed} and lines are fitted by first order kinetic model.

The influence of pH on B_0 and k_{hyd} is shown in Fig 2.4. Degradability was significantly influenced by the batch set ($p = 0.006$), with batch 2 consistently having 20% lower degradability

than batch 1. This could be due to seasonal factors where batch 1 samples were collected in winter and batch 2 in summer. The chemical characteristics of these samples are shown in Table 2.1. Because of different sampling times, the VS_{fed} of WAS in batch 1 and 2 were 0.935 g L^{-1} and 0.774 g L^{-1} respectively. However, for both batch 2 and batch 1, low pH (<5.7) caused a decrease of 33% degradability compared to neutral pH (>7), ($p = 0.004$). Higher reductions (on the order of 64%) have been observed for sludge digestion (Lay, Li et al. 1997). There were no significant interaction effects between batch number and pH on either rate or extent of degradation. Hydrolysis coefficient was higher for batch 2 ($p = 0.01$), but was not influenced by pH ($p = 0.12$) as shown in Fig. 2.4. The low pH conditions influenced conversion extent to methane, but not rate of methane production contradicting the standard assumption in biochemical process modelling, that pH will decrease rate, but not the amount of material able to be degraded (Batstone, Keller et al. 2002). This was largely due to the accumulation and decreased degradation of particulate organic matter (POM) at low pH (pH <5.7). Identification of POM as un-degraded fraction was based on mass balancing, with only a small fraction (Fig. 2.5) being due to increased soluble COD or VFA. Reduced methane potential has been previously reported at depressed pH conditions (Lay, Li et al. 1997), and Gomec and Speece (2003) particularly noted that the effect of reduced pH on WAS was reduced hydrolysis, while reduced pH on primary sludge caused an accumulation of organic acids and soluble. A decrease in observed methane potential at low pH has been previously reported (50% loss at pH 5.25) (Chen, Jiang et al. 2007). CO_2 content in the biogas varied with pH, it was recorded 58%, 53%, 41%, 44%, 48%, and 34% at pH 5.25, 5.6, 6.2, 6.4, 6.9, and 7.7 respectively.

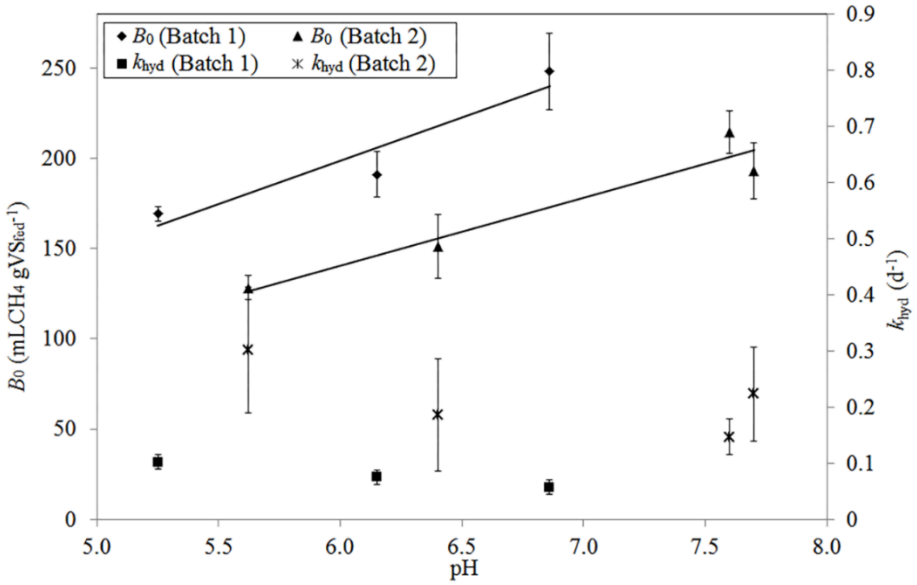

Figure 2.4: Effect of pH on degradability, B_0 ($mLCH_4$ gVS_{fed}^{-1}) and hydrolysis rate, k_{hyd} (d^{-1}) for the batch anaerobic digestion of waste activated sludge. Linear regression lines represent the possible trend of B_0 and k_{hyd} at each pH.

No significant relation between hydrolysis coefficient and pH at a 5% significance threshold was observed. Effect of pH on hydrolysis has been widely reported in literature for various waste streams containing both primary and activated sludge, between 0.1-1 d^{-1} (Miron, Zeeman et al. 2000, Feng, Yan et al. 2009). This study also differs from the findings of Arntz, Stoppok et al. (1985) who suggested that pH 6.5 can be suitable for optimal hydrolysis while using beet pulp as substrate in AD. This increase in hydrolysis supports multi-stage anaerobic digestion, with earlier stages focusing on low pH operation (with possibly recovery of phosphorous).

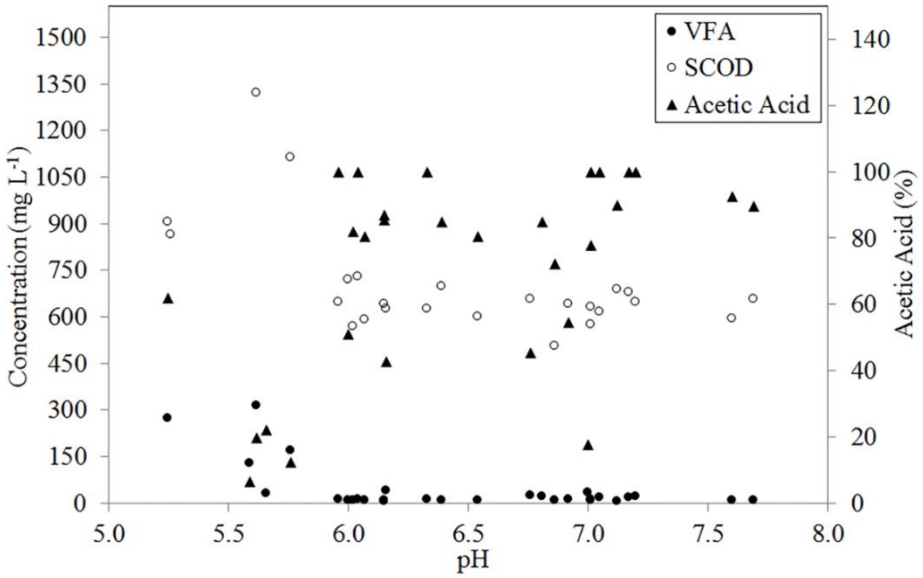

Figure 2.5: Concentration of total SCOD and total VFA produced at different pH conditions. Acetic acid as a percentage of total VFA has been shown in secondary axis.

Table 2.3: Individual VFAs as percentage of total VFA at major pH values of both batch tests.

pH	Total VFA	HAc (%)	HPr (%)	HBu (%)	HVa (%)	HHe (%)
5.3	273	62	13	10	15	1
5.6	312	20	53	3	23	2
5.7	30	22	18	13	13	34
5.8	168	12	64	0	24	0
6.1	7	81	11	9	0	0
6.2	5	87	0	0	13	0
6.4	9	85	7	0	8	0
6.5	9	80	11	0	8	0
6.8	23	45	27	6	13	9
6.9	9	72	6	1	7	13
7.0	7	78	11	0	11	0
7.1	6	90	0	0	10	0
7.6	7	93	1	0	7	0

VFA and SCOD concentrations remained constant for the pH range 6.2-7.6 but were increased at pH below 6.2 as shown in Fig. 2.5. The highest SCOD concentration (1320 mg L^{-1}) was observed at pH 5.62 while lowest SCOD concentration (576 mg L^{-1}) was at pH 7 ($p = 0.006$). As shown in Fig. 2.5, the majority of the SCOD was VFA, with the majority of this being acetate (Table 2.3). This supports that inhibition at low pH results in residual concentrations rather than a decrease in conversion rate. The increase in SCOD resulting from hydrolysis and fermentation was fastest during start up after which residual VFAs were reduced.

Acetic acid was found at all test conditions whereas propionic and butyric acids were exclusively found at depressed pH conditions (pH <6.2). The total VFA concentration was 273 mg L^{-1} at pH 5.25 and 312 mg L^{-1} at pH 5.62 ($p = 0.001$). Although VFAs were accumulated at depressed pH conditions, the anaerobic process and methane production was stable which means the fatty acids might be continuously converted into acetate albeit with residual concentrations (Zoetemeyer, Van den Heuvel et al. 1982).

2.3.2 Community profiling

All samples were dominated by *Methanosaeta* (Fig. 2.6), with 40%-80% of total sequences in affiliated operational taxonomic units (OTUs) which states that decrease in methane potential was mainly due to the decreased hydrolytic capacity, thus, indicating that principal methanogen (*Methanosaeta*) was not affected. However, this was not influenced by pH ($p = 0.77$), and because it obscured other results, the *Methanosaeta* OTUs were removed, and the OTU table was re-normalized and analyzed. Based on principal component analysis (PCA – excluding *Methanosaeta*) of samples at pH 5.25, 6.15 and 6.86 (batch 1) and 5.76, 5.96, 7.05, 7.17 (batch 2) along with inoculum found that first two PCs represented the majority (64%) of variance (Fig. 2.7). Batch number did not affect PC 1 ($p = 0.4$) or PC 2 ($p = 0.11$) but did have an impact on PC3 ($p = 0.02$).

Overall, pH was the primary driver of community (Fig. 2.7), with an influence mainly on PC1 ($p = 0.03$), but not PC2 (0.06) or PC3. When inoculum was also considered, the evidence of impact was even higher ($p = 0.004$). Therefore, both batches were affected by pH, with consistency also from inoculum. The shift in microbial community was mainly due to a shift from *Clostridium sp., Levilinea sp.* and *Nocardioides sp.* at low pH (pH 5.25 and 5.76), *Methanoculleus sp.* was at moderate pH 6.15, and *Methanobrevibacter sp., Candidatus Cloacamonas acidaminovorans str., Methanospirillum sp.* and *Methanoculleus sp.* at high pH 7.2 (inoculum). Inoculum appeared to cluster with other high-pH

samples with inoculum lying to the far right of the pH correlated PC1. *Bacillus Aquimaris* was the next dominant OTU, and was generally suppressed as pH decreased below 7.0. Therefore, overall pH decrease caused a loss in diversity in the primary hydrolytic community with a shift towards *Clostridium* from multiple bacterial hydrolytic candidates and a shift within the hydrogen utilizing community towards *Methanoculleus* from *Methanobrevibacter* and *Methanospirillum*. The chemical and BMP analysis identifies a reduction in hydrolytic extent as the main cause of reduced potential. The microbial results support this, with the main impact of low pH operation being related to bacterial (hydrolytic) shift rather than changes in methanogenic archaea.

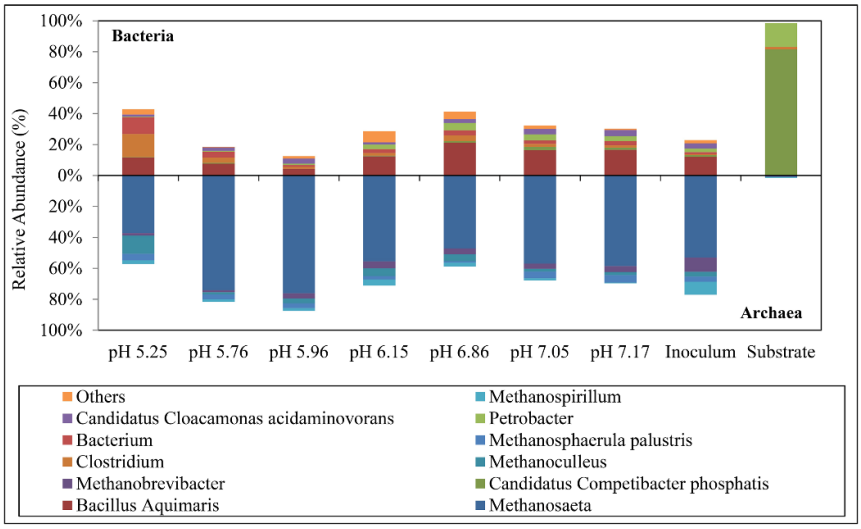

Figure 2.6: Relative abundance of microorganisms at various pH conditions in both batch tests

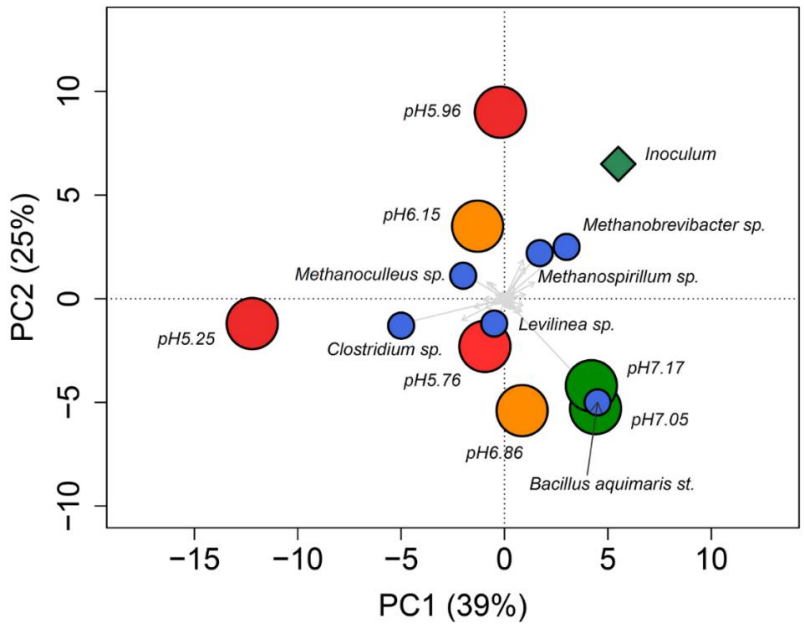

Figure 2.7: Principal component analysis biplot at each pH at 35 days of batches 1 and 2. Orange and red circles represent the pH of sludge samples for batch 1 and 2 respectively. Blue circles are the vectors representing orientation of microorganisms with respect to pH. Green box is showing the inoculum of batch 2 only.

2.3.3 Effect of pH on PO_4 and cations concentration

Analyzing all sacrificed bottles, and end points, a strong correlation ($p = 0.0005$) was found between pH and PO_4 (Fig. 2.8). Data from both batch tests is integrated to one plot, with further data provided in supplementary material. At initial conditions (pH 6.8), the total P concentration in each BMP bottle was the same, 1060 ± 90 mg L^{-1} and PO_4 concentration was 215 mg L^{-1}. During the BMP test, highest PO_4 (799 mg L^{-1}) was observed at pH 5.25 (75% of the total P), while at neutral pH it was around 200 mg L^{-1}. Consequently 57% increase in PO_4 was observed at pH 5.25 compared to neutral conditions (pH 7.0 or above) where the PO_4 concentrations remained close to initial conditions. This substantially expands the range of analysis previously observed (Mehta and Batstone 2013) and indicates that almost all P can be available as PO_4, if a suitably low pH is selected. Current findings are higher than previous study by Bi, Guo et al. (2012) who observed 25% increase in PO_4 concentration at pH 5.0 (P 168 mg-$P_{soluble}$ L^{-1}) compared to pH 10 (213 mg-$P_{soluble}$ L^{-1}) in a 20-days batch AD of WAS where they started AD directly from pH 5 and 10.

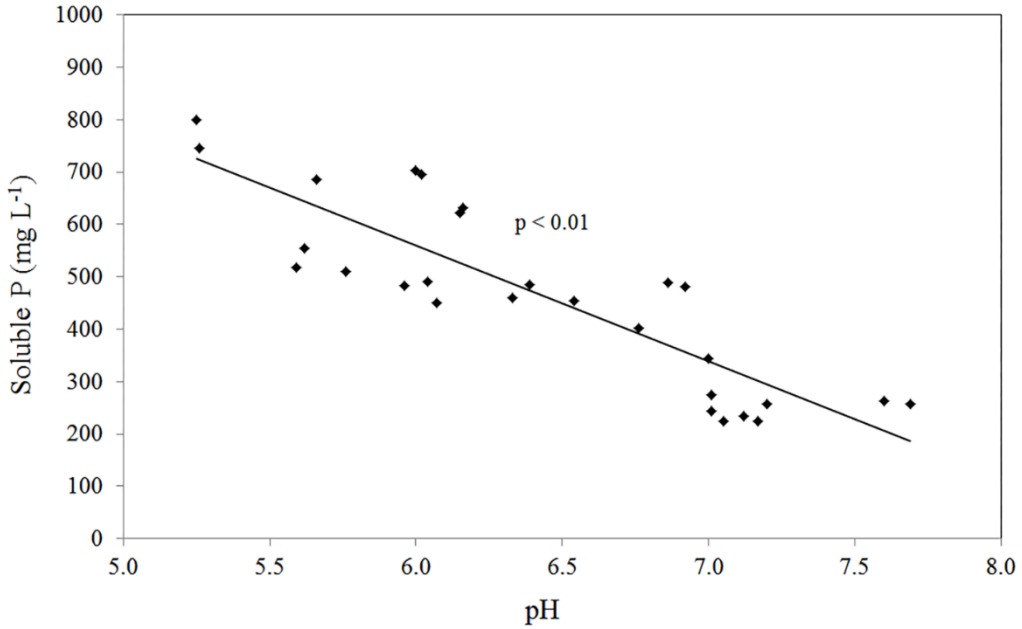

Figure 2.8: PO_4 concentration during anaerobic digestion of waste activated sludge at different pH values. Linear regression line shows the possible trend of P release with decreasing pH.

The increased solubility of P can be related to the dissolution of Ca-P and Mg-P compounds under acidic conditions (Fig. 2.9). For the batch test, total calcium and magnesium concentrations were 560 and 273 mg L^{-1} respectively. The soluble calcium concentrations at pH 5.6 and 7.7 were 244.6 and 30 mg L^{-1} respectively. Similarly, the soluble magnesium concentrations at pH 5.6 and 7.7 were 262 and 7 mg L^{-1} respectively thus eliminating the possibility of struvite formation as almost

whole magnesium was released at this stage. However, there could be a chance of the formation of calcium phosphates, as 53% of calcium was not released even at low pH (pH 5.6). Therefore, a post treatment could be required to completely release calcium for increasing PO_4 concentration in effluent. These results are comparable with the findings of (Jardin and Popel 1994) who also found a linear relationship of calcium release with P but differ from the results of magnesium release with P release.

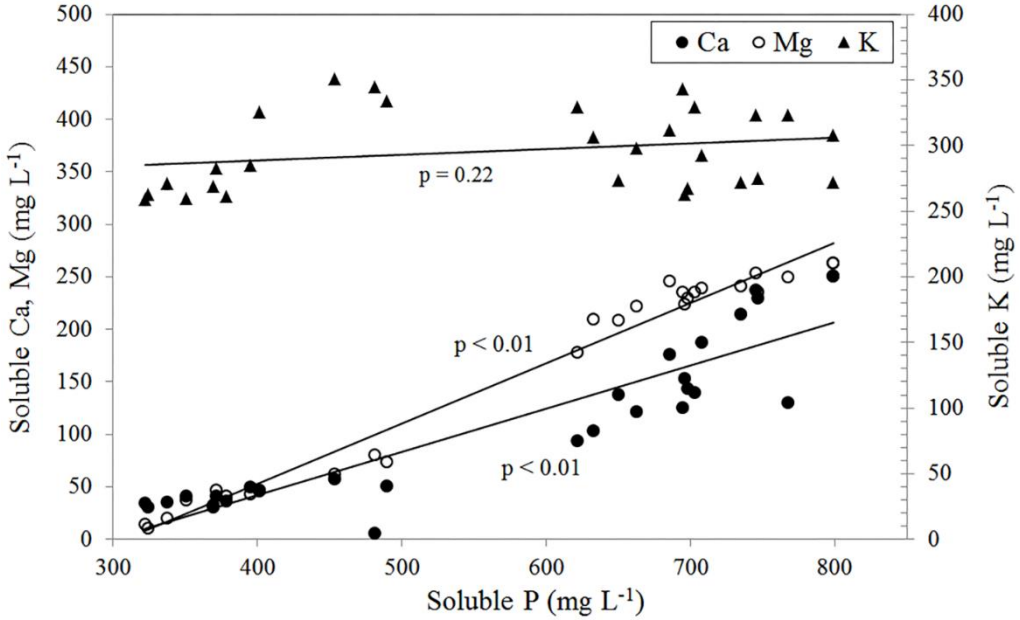

Figure 2.9: Correlation between PO_4 and Ca, Mg and K. Linear regression lines show the possible trend of cations release with released P.

2.4 Conclusions

Phosphorous release was increased up to 3.6 times under acidic conditions with a 33% reduction in methane yield compared to neutral conditions. Reduction in methane potential at low pH was mainly due to reduced hydrolysis of particulate organic matters, rather than an increase in soluble organics. *Methanosaeta* dominated in general, and was not influenced by pH, but pH caused a shift and narrowing in bacterial diversity towards *Clostridium* and within the hydrogen utilizing methanogens towards *Methanoculleus*. Low pH is a suitable option for enhanced phosphorous release, but work is needed to realize a chemical free option.

3. INFLUENCE OF LOW pH ON CONTINUOUS ANAEROBIC DIGESTION OF WASTE ACTIVATED SLUDGE

3.1 Introduction

In the previous chapter, low pH batch AD identified a significant increase in PO_4 concentration, but with a major loss in methane potential. It was underscored that the negative issues with low pH operation were acid requirements, accumulation of volatile fatty acids (VFAs), loss in methane production, and inhibition of methanogens. These outcomes may change with continuous operation, due to acclimatization of microbial community (Chen, Cheng et al. 2008). Batch testing does not allow acclimatization of inoculum, and the required periodic dosing of acid during a batch process represents both short and long term disruptions, particularly in batch reactors operated at low pH (< 5.5). In this chapter, low pH conditions in a single stage continuous AD process were studied using bench scale reactors with a continuous acid dosing, to mimic the full-scale process, and enable determination of longer-term shifts.

Redrafted after

Muhammad A. Latif, Chirag M. Mehta, Damien J. Batstone. (2017). "Influence of low pH on continuous anaerobic digestion of waste activated sludge." Water Research 113: 42-49.

3.2 Material and Methods

Three identical laboratory scale continuous anaerobic digesters were operated with one as neutral (pH 7.0) control, and the others operated at pH 6.5, 6.0, 5.5 and 5.0, fed with a diluted waste activated sludge. Methane production, VFAs, solid concentrations, microbial community, PO_4 and cation concentrations at each pH condition were monitored in this study.

3.2.1 Wastewater collection

Waste activated sludge (WAS), the substrate for AD was collected from a full-scale sewage treatment plant located in Brisbane. Further details on the treatment plant and its operation, sample collection and storage are provided in Chapter 2, Section 2.2.1. The substrate was collected 7 times (once a month) over the experimental period (202 days). The substrate was sieved and diluted 50:50 with tap

water to minimize clogging in the feed lines. No major variation in the physio-chemical properties was observed in the collected 7 samples, as shown in Table 3.1.

Table 3.1: Physico-chemical characteristics of the diluted waste activated sludge. Values are given as mean ± confidence interval for seven samples collected during the course of the experimental period (202 days).

Parameter	**Total (g L^{-1})**	**Parameter**	**Total (mg L^{-1})**	**Soluble (mg L^{-1})**
pH*	6.3 ± 0.1	P	540 ± 20	124 ± 3 (PO_4-P)
COD	23 ± 0.8	Mg	172 ± 6	68 ± 1
TS	19 ± 0.8	Ca	237 ± 12	60 ± 17
VS	15 ± 0.5	K	214 ± 15	89 ± 3
		Na	321 ± 8	293± 5
		Fe	154 ± 13	3 ± 1
		Al	76 ± 7	-
		N	337 ± 7	34 ± 7 (NH_4-N)

*unitless parameter

3.2.2 Anaerobic reactor setup and operation

Three identical 1.5 L jacketed glass reactors (two tests and one control) were connected using Masterflex® L/S® peristaltic pumps (model 7553-89, Cole-Parmer, USA) with multi-heads for simultaneous feed and drain. The process was controlled at 37 ± 1 °C by supplying heated water to the jacketed reactors. The continuous stirred tank reactor contents were mixed (at 250-300 rpm) using magnetic stirrer plates. The reactor pH was controlled automatically using programmable logic control (PLC) supplied by Direct Automation, Australia, and manually (when required) using 1 M hydrochloric acid (HCl). The pH of the test reactor was controlled by 1 M HCl, dosed by a small peristaltic pump operated by the PLC (Fig. 3.1). Biogas production was measured by custom made bucket type water filled gas meters (2.25-2.5 mL/bucket) connected to the PLC. A schematic diagram of the reactor setup is shown in Fig. 1, which includes accessories and setup as discussed above.

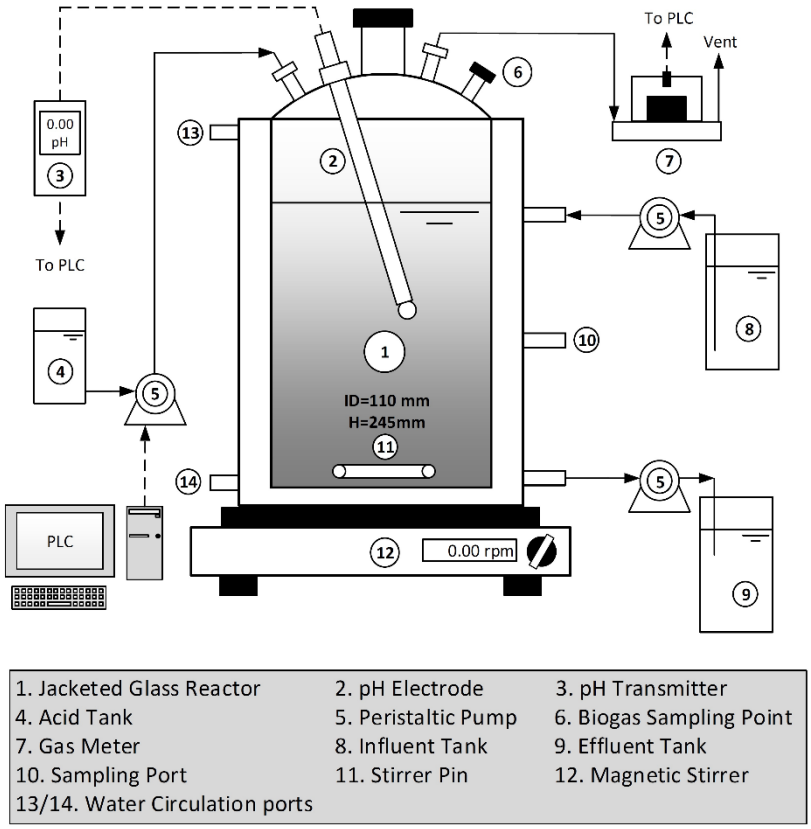

Figure 3.1: Schematic diagram of low pH continuous anaerobic digestion process, associated tanks, pumps, instrumentation, control unit and flow direction.

Initially, the reactors were seeded with 1 L of assay containing 365 mL of the substrate (5.81 gVS added) and 635 mL of the inoculum (9.41 gVS added) by maintaining an inoculum to substrate ratio (ISR) of 1.62 on VS basis (wt%). The pH of the assay was set to 7.0 using 1 M sodium hydroxide (NaOH) solution. Each reactor was purged with nitrogen gas for at least two minutes to provide anaerobic conditions. The reactors were operated using a 12-day hydraulic retention time (HRT), having an OLR of 1.91 ± 0.04 gCOD $L^{-1}.d^{-1}$ and a flow rate of 83 ± 2 mL d^{-1}. The test reactors were initially operated at control conditions (Fig. 3.2) and were adjusted to the required pH once steady state conditions were achieved, i.e. the performance parameters (methane yield, VS and COD removal) of the test reactor were similar to the control reactor. The data represented in all sections has been interpreted as control (pH 7.0), pH 6.5 (test reactor 1), pH 6.0 (test reactor 1), pH 5.5 (test reactor 1) and pH 5.0 (test reactor 2). Note, the test reactor 2 was added later (at day 99) and was adjusted to pH 5.0 after four weeks of operation at control conditions (Fig. 3.2). At each pH, the reactors were operated for at least 4 HRTs (48 days), while the data was reported for last 2 or 3 HRTs.

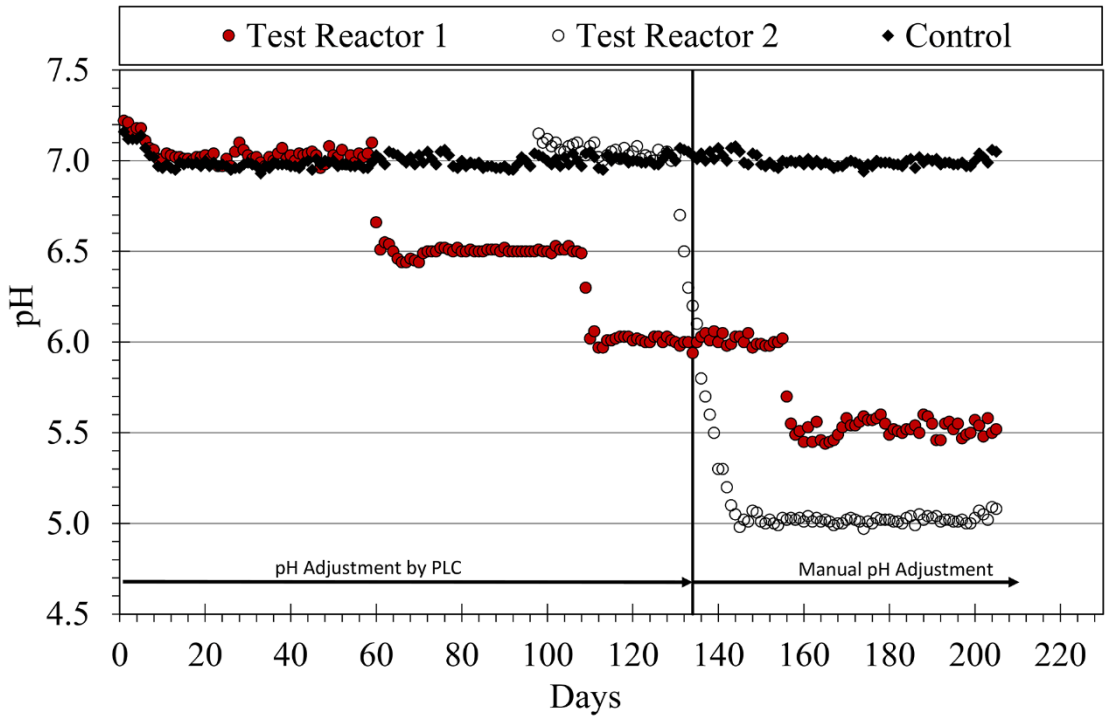

Figure 3.2: Daily pH profile of all reactors and the pH adjustment schedule of test reactors. An automatic pH adjustment was carried out until 135 days, and it was done manually afterwards.

3.2.3 Analytical techniques

Total solids (TS), VS, COD, SCOD, VFA and, elemental concentrations and microbial analysis were measured according to the protocol given in Latif, Mehta et al. (2015). Biogas composition (CH_4 and CO_2) was measured using a Shimadzu GC-2014, Japan gas chromatograph (GC) equipped with a Valco GC valve (1 mL sample loop), a HAYESEP Q 80/100 packed column (2.4 m length; 1/8" outer diameter, 2 mm inner diameter) and a thermal conductivity detector (TCD). The chromatograph injector, oven, and detector temperatures were set at 75, 45 and 100 °C, respectively, and 28 mL min^{-1} of argon at 135.7 kPa was used as a carrier gas. The GC was calibrated using external gas standards obtained from British Oxygen Company (BOC). Pyrosequencing was done on the sludge samples collected at pH 7.0, 6.5, 6.0, 5.5 and 5.0. The samples for the pyrosequencing were collected just before applying the new pH conditions. Total DNA was extracted from sludge samples using FastDNA® Spin Kit for soil (MP Biomedicals, California, USA).

3.2.4 Residual biochemical methane potential

Residual methane potential was performed on the digested sludge (digestate) recovered from the test and control reactors using a modified biochemical methane potential (BMP) protocol (Jensen, Ge et

al. 2011). The purpose of this test was to assess the capacity of sludge for additional methane production. The digested sludge samples were stored at 4 °C, prior to use in the BMP test. The working volume of each BMP was 100 mL. The pH was adjusted to 6.9 ± 0.1 using 1M HCl or 1M NaOH solution prior to start the test. Each sludge sample was tested in triplicates for 40 days at 37 ± 1 °C. The test was performed in two sets, based on the type of inoculum and sample: (1) native inoculum, effluent sample (NI) consisted of 100mL experiment and control pH digested sludge collected from the test reactor at different pH conditions (pH 5.0, 5.5, 6.0, and 6.5), as well as the 7.0 control and; (2) control inoculum, effluent sample (CI), which included 50 mL of the low pH digested sludge (pH 5.0, 5.5, 6.0, and 6.5) and 50 mL of the control effluent (pH 7.0). The rationale for this was to test both terminal methane yield using low pH sludge and to amend the inoculum in the CI experiments. For the CI set, the true methane yield (L $kgVS_{fed}^{-1}$) was estimated by subtracting half of the methane yield for the control inoculum in its NI equivalent (i.e., the 100 mL test at pH 7.0), in order to account for the 50 mL added as inoculum. The B_0 values presented for CI experiments therefore represent the calculated contribution of the effluent sludge only and should be directly comparable to the NI results. The data was compared by degradability (B_0) and first order hydrolysis coefficient (k_{hyd}) as previously described in Latif, Mehta et al. (2015).

3.2.5 Acid digestion test

A modified acid digestion test (ADT) was performed on the digested sludge to estimate P fraction as a soluble (as PO_4), total inorganic (precipitated P) and organic (bound with nucleic acids) (Mehta and Batstone 2013). The test was conducted using a 50 mL digested sludge samples from each pH condition, stirred at 300 rpm using a magnetic stirrer bar. The pH of the samples was recorded and adjusted to 2.5 ± 0.1 using 3 M HCl solution. The acidified slurry was stirred for two hours to ensure complete solubilization of precipitants followed by another pH adjustment to 2.5 (if required), and further stirring for 24 hours followed by sampling for elemental analysis. Each sludge sample was tested in triplicates. The total inorganic P in the sample was assumed to be the difference between PO_4 concentrations before and after the ADT test, while the organic P was assumed as the remaining P in the sludge sample following ADT (organic P = total P in the sludge – PO_4 after ADT).

3.2.6 Statistical Analysis

All errors (±) and error bars are 95% confidence in mean using a two-tailed *t*-test (5% significance threshold). Linear correlation was assessed in Microsoft Excel 2010 using the regression tool with reported *p*-values being for the standard linear model. Parameter uncertainty for non-linear regression

is 95% confidence using a two-tailed *t*-test, with parameter standard error estimated from the Fisher information matrix as described in Latif, Mehta et al. (2015). Where derived values are analytically calculated from primary observed values, errors are propagated analytically (Batstone 2013).

3.3 Results and discussion

3.3.1 Reactor performance

Methane production (L-CH_4 $kgVS_{fed}^{-1}$) was consistent at each pH condition after the initial adjustment period as shown in the Fig. 3.3b, which was typically less than a week. No transient inhibition in the methane production was observed at reduced pH conditions, as reported in literature (Jain and Mattiasson 1998, Taconi, Zappi et al. 2008). The reported transient inhibition was likely due to step dosing of acid causing pH shock, while in this study, the pH was gradually changed over 24 h. Average methane yield (L-CH_4 $kgVS_{fed}^{-1}$), VS destruction (%) and COD removals (%) at each pH condition are shown in Fig. 3.3a. The average methane yield for the control reactor (pH 7.0) was 69.3 ± 1.2 L-CH_4 $kgVS_{fed}^{-1}$ (n = 202). This value is significantly lower than the reported values (>150 L-CH_4 $kgVS_{fed}^{-1}$) for the same substrate at 12 days HRT (Lafitte-Trouqué and Forster 2002, Lee, Parameswaran et al. 2011). This could be due to a relatively low hydrolysis coefficient (k_{hyd} = 0.035 d^{-1}, see Table 3.2) causing reduced solubilization of the particulate organics from the WAS, and hence lower overall methane conversion. The average methane yield and methane content in biogas reduced linearly with decreased pH conditions and the correlation was significant (p = 0.001, n = 36). The methane content in biogas was 65, 57, 51, 47, and 41% at pH 7.0, 6.5, 6.0, 5.5, and 5.5 respectively. The poor methane yield was reflected in reduced VS destruction and COD removal as shown in Fig. 3.3a. The CO_2 content in biogas increased with acidic pH conditions, averaging of 36%, 38%, 44%, and 46% of CO_2 at pH 6.5, 6.0, 5.5, and 5.0 respectively (Appendix 3.1). The influence of pH on these measures was significant, and the p-values by regression were found as 0.008 and 0.004 for COD removal and VS destruction respectively.

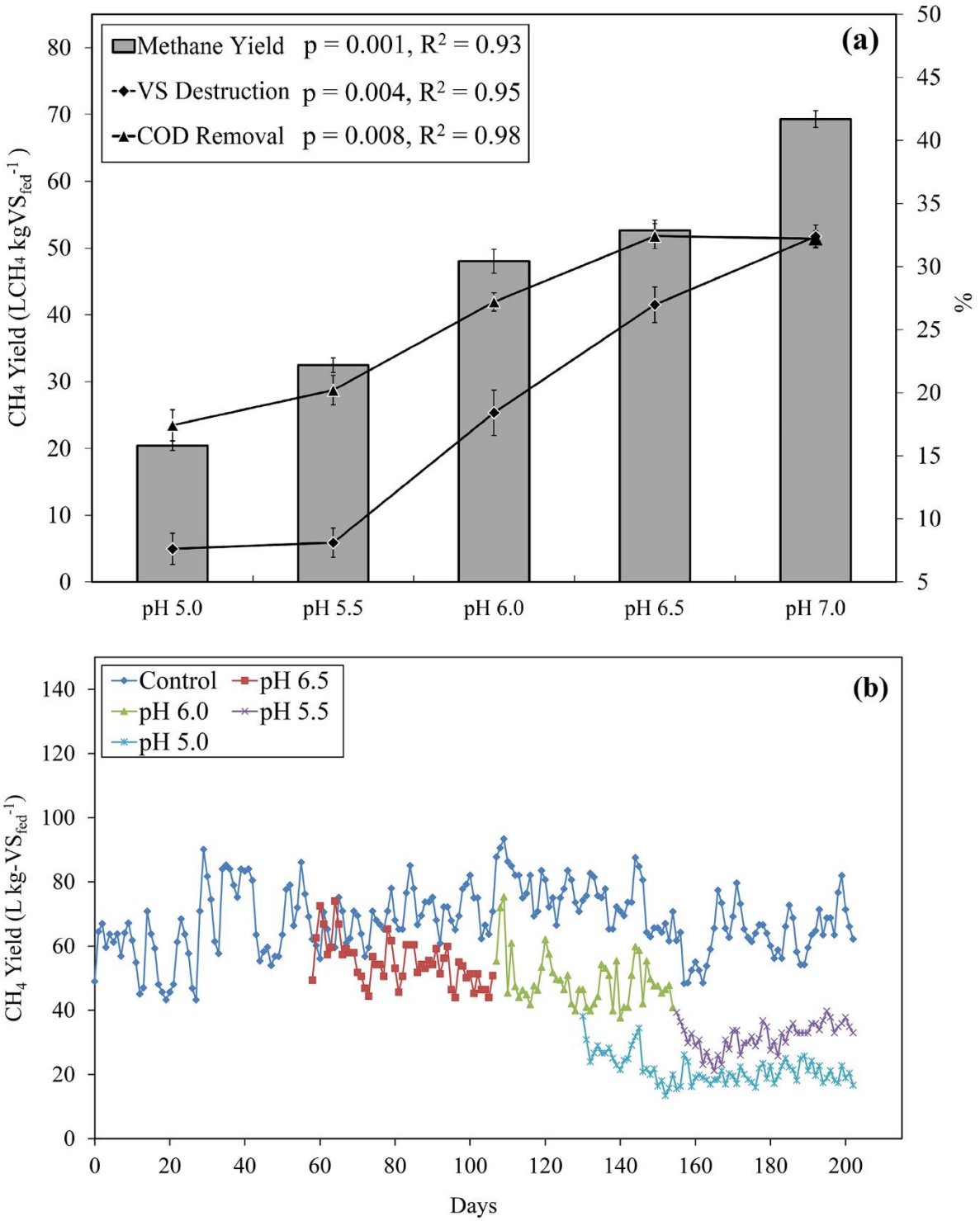

Figure 3.3: (a) Methane yield at each pH during whole experimental period. (b) Average methane yield, VS destruction and COD removals at various pH conditions. Error bars are 95% confidence intervals (CH_4: n = 36, VSD and COD removal: n = 15).

Table 3.2: Model based determination of the hydrolysis rate coefficient of continuous reactors.

pH	k_{hyd}
5.0	0.011
5.5	0.027
6.0	0.027
6.5	0.035
7.0	0.035

Note: the hydrolysis coefficient was calculated from $y_d = (1 – 1/1+kt)$ (Ho, Jensen et al. 2013),
Where, $y_d = B_c/B_b$, a fraction calculated by the observed methane from the continuous operation (B_c) and BMP test (B_b). The values of B_b were taken from (Latif, Mehta et al. 2015).

Fig. 3.4 shows the total SCOD, total VFAs concentration and VFA types at the different pH conditions. The total SCOD and VFAs concentrations were below 200 mg L^{-1} and 20 mg L^{-1} respectively, at pH 6.0, 6.5 and control, suggesting stable methanogenesis. Reduced pH conditions (pH < 6) showed a large increase in the total SCOD and VFAs concentration, with increased concentration of propionic acid and valeric acids. Further analysis of the apparent hydrolysis rate (Table 3.2) indicated a relatively low in-reactor hydrolysis coefficient on the order of 0.05 d^{-1}, decreasing as pH decreases to <0.03 d^{-1}. Further, the trends in VFA concentration shows that acetogenesis is also substantially inhibited at a pH<5.5, with strong accumulation of the higher VFA levels, including propionic, butyric and valeric acids. High propionic acid concentration (367 ± 10 mg L^{-1}) and propionate/acetate (P:A) mass ratio was also recorded in the current study, and the P:A was found as 27 ± 1.5 and 32 ± 1 at pH 5.5 and 5.0 respectively (n = 7). Such high values correlate with process instability and impending digester failure (Hill, Cobb et al. 1987, Ahring, Sandberg et al. , Pullammanappallil, Chynoweth et al. 2001), in general, the key limiting factors appeared to be hydrolysis, and acetogenesis, rather than methanogenesis.

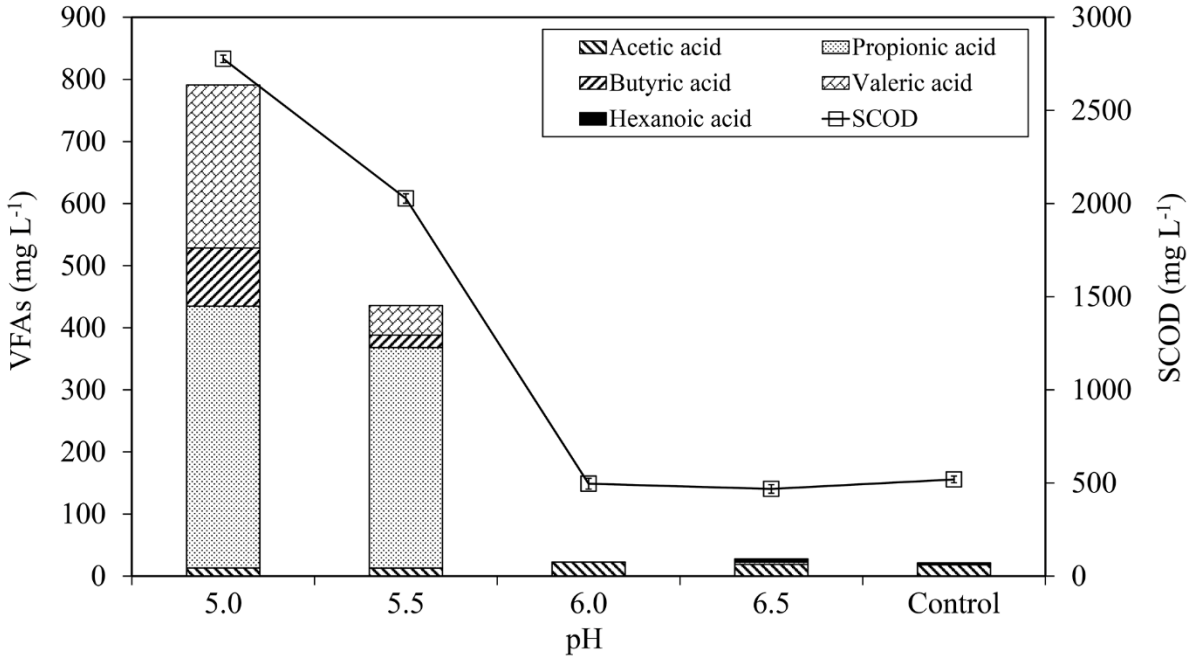

Figure 3.4: Average total and individual VFAs along with soluble COD at each condition. Error bars are 95% confidence intervals.

3.3.2 Residual methane potential

The B_0 and k_{hyd} of the control, NI and CI experiment are given in Table 3.3. Methane yield on NI experiments was very similar (100-120 L CH_4 kg-VS_{added}), while significantly higher overall yield was observed for CI experiments. Higher degradability of the BS samples was due to a combination of high concentration of VFAs in the low pH digestate (substrate) and enhancement of the methanogens provided by added inoculum (control). The total methane production (continuous + batch) of the control sample was 187 ± 5 L CH_4 kg-VS_{added}, which was similar to the previous batch study on similar substrate (Latif, Mehta et al. (2015). This indicates potential for a two-stage process, in which the first stage operates at low pH digestion, followed by stage II at neutral pH. A significantly higher k_{hyd} was also observed for CI experiments ($p = 0.025$ for single-tailed paired t-test).

Based on these results, and comparing NI and CI experiments, further analysis could be made of different fractions on a VS basis (%VS removed) at different pH conditions (Fig. 3.5). This presents in-digester VSD (lower bar), that attained subsequently in CI experiments (central bar), and residue (top bar), which are analogous to 1st stage, second stage (infinite HRT), and non-degradable, respectively. This indicates that while the low pH continuous reactors never reach the VS destruction in the control (pH 7) reactor, a subsequent step can enable substantial further VS destruction. Specifically, operating the first step at pH 6.0 or 6.5 enables access to material that is non-degradable

in the control, or in the one-step feed BMP. This appears to support the performance of technologies such as acid-gas sludge digestion, in which a two-stage or multi-stage process is deliberately operated with a low pH in the first stage (Parry 2012). However, our results indicate benefit at pH 6.0 and particularly 6.5, with no benefit at 5.0 or 5.5 in the first stage. Our results also support the use of a two stage AD process in a nutrient recovery perspective, where nutrients can be solubilized in the first stage via low pH, and methane loss can be compensated in the second stage via neutral AD. Since the pH decrease in the first stage is moderate (e.g., 6.0-6.5) this may be achieved through natural acidification, particularly where co-substrate is present.

Table 3.3: Model based analysis of the biochemical methane potential of control and low pH digestates, and the feed sludge[1].

Sludge	**Native Inoculum**		**Control Inoculum**	
	B_0 **(L-CH_4 g-VS_{added}^{-1})**	k_{hyd} **(d^{-1})**	B_0 **(L-CH_4 g-VS_{added}^{-1})**	k_{hyd} **(d^{-1})**
Feed[2]	220.0 ± 5	0.19	-	-
pH 5.0	130.4 ± 4.6	0.07	175.9 ± 1.7	0.10
pH 5.5	119.2 ± 3.3	0.08	144.8 ± 3	0.11
pH 6.0	114.2 ± 5.6	0.04	156.7 ± 5.5	0.05
pH 6.5	110.9 ± 1.2	0.05	168.7 ± 1.2	0.06
pH 7.0	115.3 ± 2.1	0.03	-	-

[1] k_{hyd} is the first order hydrolysis coefficient; B_0 is the degradability extent notated as methane potential. Digestate samples for the RBMP were collected during last week of each pH condition. Feed sample was the sludge being fed into each reactor. Feed B_0 is the total degradability, while B_0 of rest of the samples is the residual degradability of the digestates in a 40-day time period. [2] values are derived from (Latif, Mehta et al. 2015).

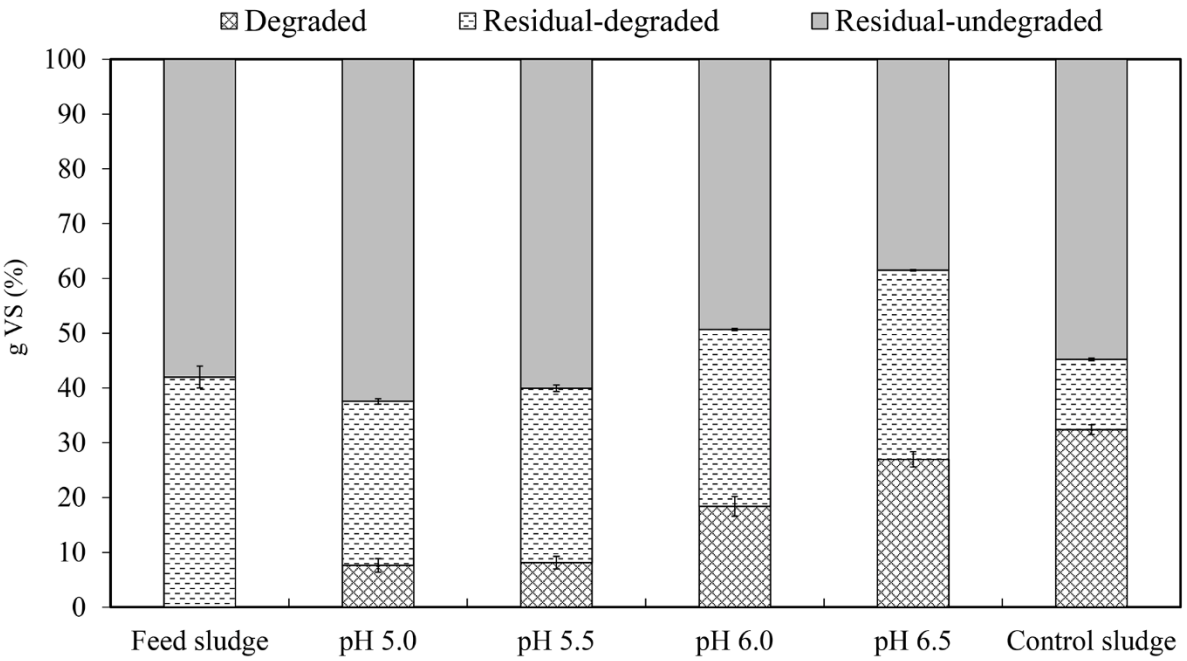

Figure 3.5: Assessment of degradable fractions of feed sludge (WAS) and digestates at different pH conditions are shown based on gram VS destroyed. Degraded fractions were measured from the continuous reactors at each pH, while residual-degraded fractions were taken from the BMP experiment of the blended sludge samples. Residual-undegraded was the biomass which was not converted into methane. Both degraded and residual-degraded fractions are represented as mean ± 95% CI of triplicates.

3.3.3 Microbial community

Fig. 3.6 shows the principal component analysis (PCA) biplot, with PC1 and PC2 representing 75% and 14% of the variance respectively. A minor shift was observed in microbial community of the control, pH 6.5 and 6.0 reactors, sampled at day 50, 106 153 and 202. At these pH conditions, the most abundant bacterial populations at the phylum level were *Proteobacteria*, *Bacteroidetes*, and *Chloroflexi*, accounting for 20 ± 0.2%, 18 ± 0.1% and 20 ± 0.2% of the total bacterial community respectively. Among methanogens, genus *Methanosaeta* accounted for 80% of the total archaeal community. The control sludge has a mixture of acetate-utilizing and fermentative bacteria along with significantly lower amounts of methanogens (13 ± 0.1% methanogens of total microbial community) that helped in hydrolyzing and fermenting the particulate organic materials into organic acids followed by CO_2 and CH_4. The microbial community was significantly influenced by pH 5.5, *Bacteroidetes* and *Chloroflexi* dominated the total bacterial community (79%) (Fig. 3.7). On the other hand, pH 5.0 further suppressed the community towards *Bacteroidetes* (see Fig. 3.7). The dominance of *Bacteroidetes* shows the existence of acidogens which could not convert the complex organics into

methane. These findings are in accordance with previous reports (Sundberg, Al-Soud et al. 2013) that abundance of *Bacteroidetes* causes an increase in soluble organics. Bacteria were the dominant microbes (84-89%) and archaea were 11-16% at pH 6.5 and pH 6.0 (having *Methanosaeta* as 6-9% of total community). The proportion of archaea was reduced to 5 and 3%, while *Methanosaeta* was reduced to 1 and 0.1%, at pH 5.5 and 5.0 respectively, showing that these two pH conditions have substantially influenced the methanogens.

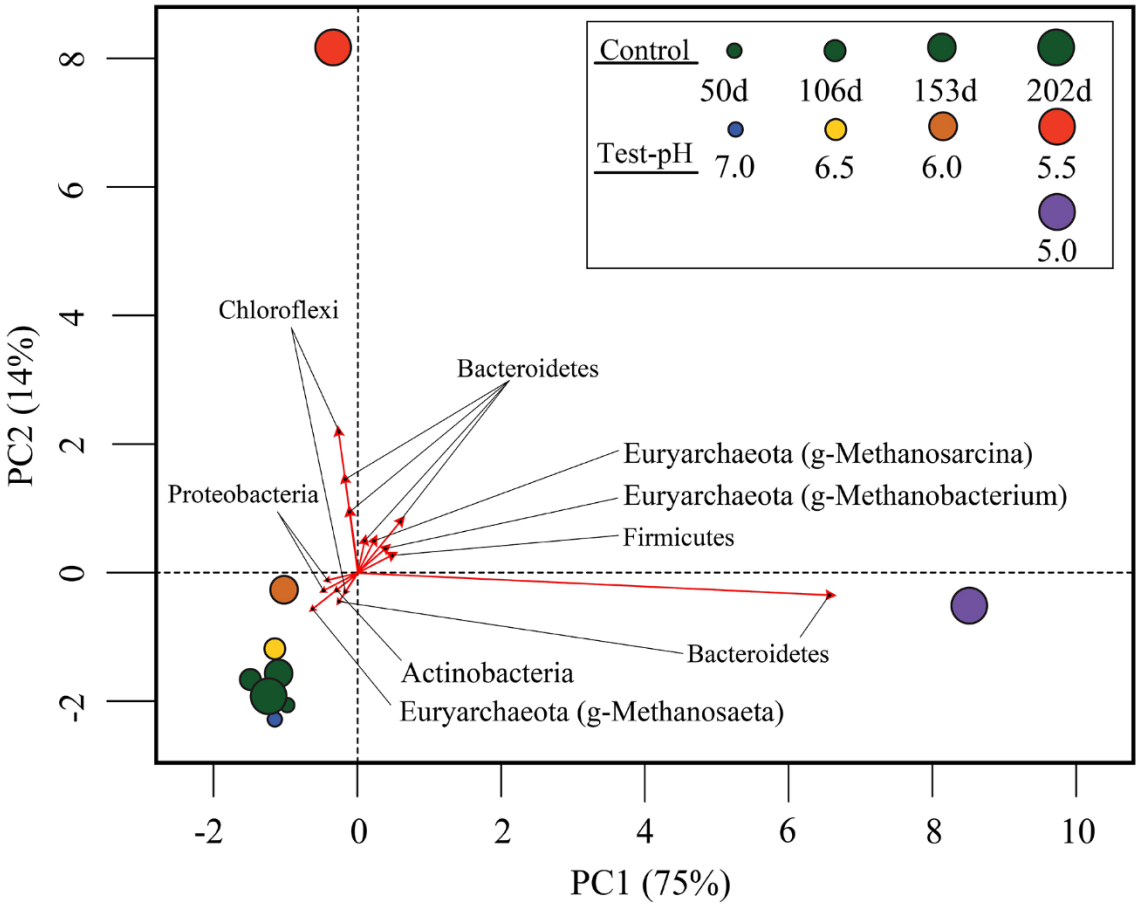

Figure 3.6: Principal component analysis biplot illustrating the phylum based microorganisms responsible for community differences at each pH condition. (a) The colored circles show the sludge sample pH, while bullets points show the distribution of microorganisms. (b) Shows the microbial diversity without pH 5.5 and 5.0. The colored circles show the distribution of microorganisms.

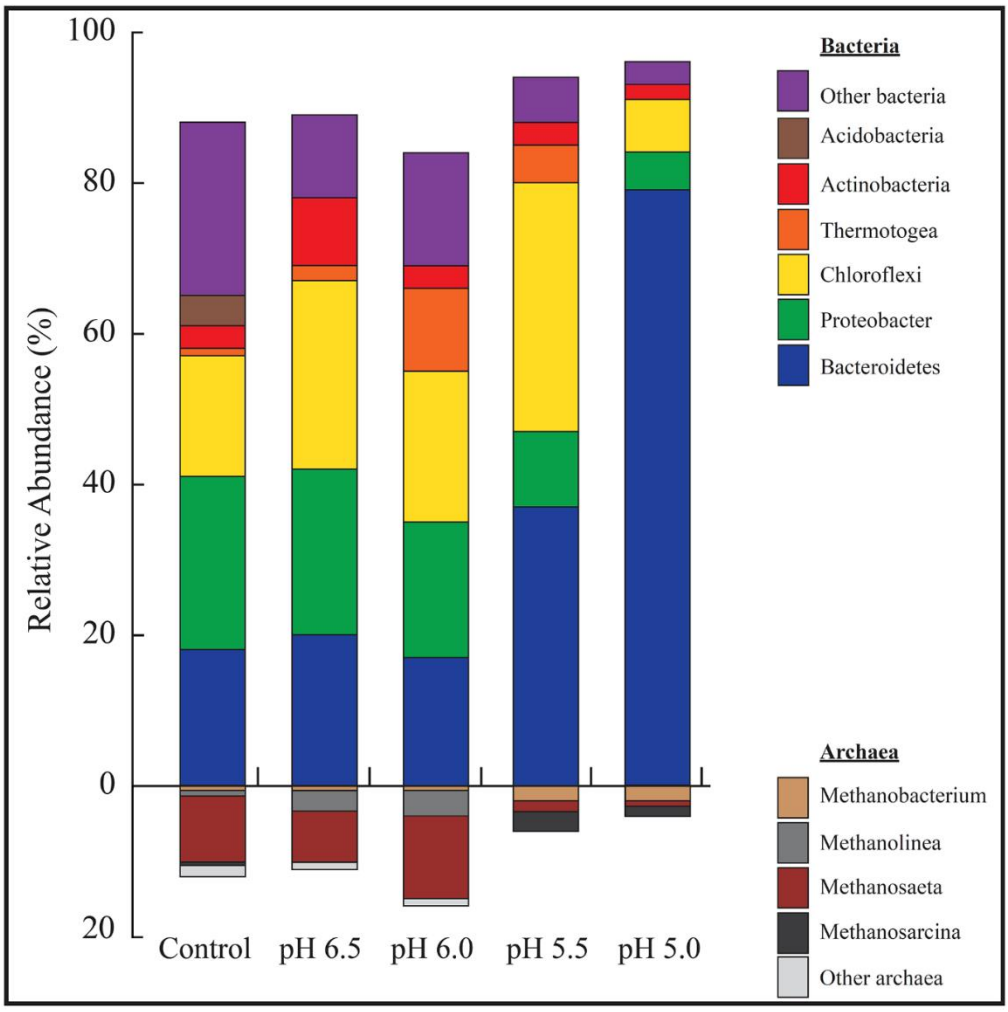

Figure 3.7: Phylum level distribution of microbial population at various pH conditions during continuous anaerobic digestion of waste activated sludge. Archaea are separated from bacteria by axis.

3.3.4 Nutrient forms and concentrations

The amount of soluble PO_4 in the digestate appeared to be dependent on pH. As shown in Fig. 3.8a, the PO_4 concentration increased proportionally from pH 7.0 to 5.5 ($p < 0.05$) and it was 261, 301, 358, and 515 mg L^{-1} at pH 7.0, 6.5, 6.0, and 5.5 respectively. No further increment in PO_4 concentration observed as the pH was reduced to 5.0, due to solubility limit. The PO_4 concentration at pH 5.5 was 74 ± 5% of the total P, while the control reactor was 42%. The PO_4 solubility trend with pH is similar, but the PO_4 concentration in the digestate was different from the previous studies (Chen, Jiang et al. 2007, Bi, Guo et al. 2012, Latif, Mehta et al. 2015), due to difference in P solubility influenced by cation type and concentration in digestate.

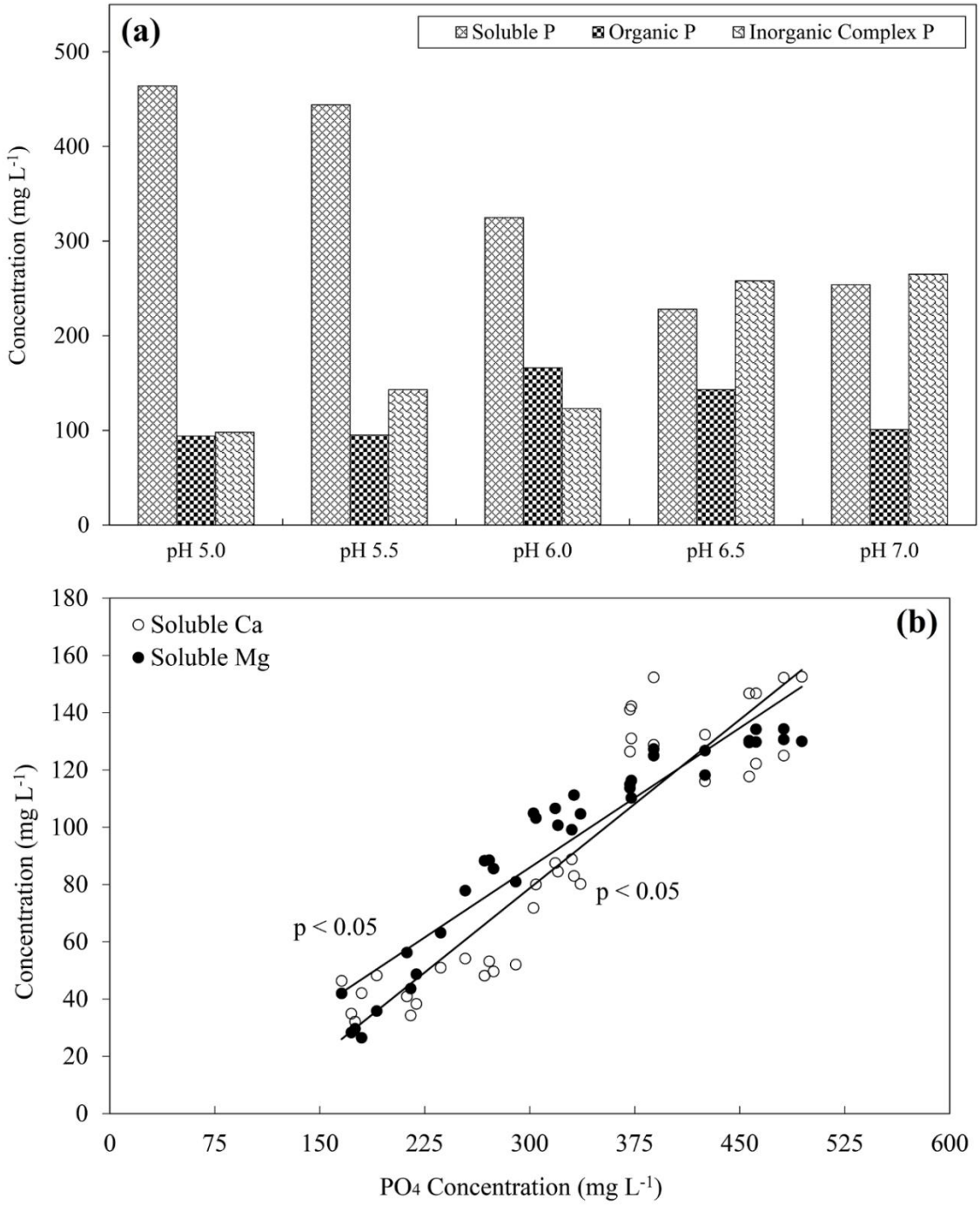

Figure 3.8: Influence of pH on nutrient form and concentration in the digestate during anaerobic digestion of waste activated sludge: (A) Soluble, organic and inorganic fractions of phosphorus, and (B) Comparison of soluble Mg and Ca with PO_4 concentrations.

As shown in Fig. 3.8a, the concentration of organic P seemed to unaffected by pH, with a minor variation at pH 6.0 and 6.5. The concentration of inorganic complex P, mainly present as P complexes with cations such as Mg and Ca, reduced as the pH was reduced. The inorganic complex P concentration was almost half of the total P concentration in the sludge at control conditions (250 mg L^{-1}). A larger portion of the inorganic complex P was solubilized at acidic pH conditions, which was

in accordance to the previous finding of (Taylor, Frazier et al. 1963). The low pH conditions triggered release of Ca and Mg from the inorganic complexes in the digestate as shown in Fig. 3.7b. The concentration of Ca and Mg increased linearly with PO_4 release ($p < 0.05$). At pH 5.5, 88 ± 2% Mg and 54 ± 5% Ca were present in soluble form; the remaining were therefore present in the precipitated or physically attached to the particulate organics. At this stage, the soluble concentration of Mg and Ca were respectively 60 and 37% higher than the neutral pH.

3.4 Conclusions

Anaerobic digestion operated at low pH conditions increased PO_4 concentration and reduced in-reactor P-precipitation. At pH 5.5, 74 ± 5.0% of PO_4 was released along with cations such as Mg and Ca, the cations responsible for in-reactor P precipitation. Methane was reduced by 50% at pH 5.5 due to the accumulation of VFAs especially propionic and butyric acids, leaving greater amount of organic matter undegraded. Optimal operational conditions for two-stage digestion appeared to be operation of the first stage at pH 6.5. The methanogenic community was significantly reduced at pH 5.0 and 5.5, with a shift towards propionate utilizing community. Additional methane was recovered from the low pH sludge following the continuous experiment, suggesting potential to recover the lost methane and further degrade the organic matter. This implies that the low pH AD technology can be implemented in a two stage process.

4. PHOSPHORUS SOLUBILIZATION VIA HIGH PRESSURE ANAEROBIC DIGESTION

4.1 Introduction

The previous chapters showed that phosphorus (P) solubilization during anaerobic digestion (AD) with low pH or complexing agents could be an expensive approach due to a higher cost of chemicals. For chemical free AD, high pressure is reported to be effective that decreases system pH by solubilizing carbon dioxide (CO_2) into the solution (Chen, Rößler et al. 2014). The in-reactor CO_2 solubility causes a reduction in the CO_2 content in the reactor headspace at high pressure, which therefore increases methane content in biogas (Chen, Rößler et al. 2014). In various studies, high pressure two-stage AD has been conducted between 2 and 90 bar pressure conditions (Lindeboom, Weijma et al. 2012, Wahidunnabi and Eskicioglu 2014) (Keymer, Ruffell et al. 2013), with a focus on either biogas upgradation or increase sludge hydrolysis. These studies found that methane content in biogas were increased up to 95% at pressure ranges from 2-90 bar, followed by a two-fold increase in methane production. However, high pressure in a single stage AD process has not been tested, especially focusing on P solubility. Therefore, the current chapter addresses this limitation of the effect of high pressure during AD on P solubility and methane production. This was performed using waste activate sludge (WAS) as substrate in a continuous single stage mesophilic operated at 1, 2, 4, and 6 bar absolute pressures. The effect of pressure on P and cations solubility, methane yield, volatile fatty acids (VFAs), solids removal, chemical oxygen demand (COD), pH, and microbiology were also reported in this chapter.

4.2 Material and Methods

4.2.1 Sample collection

The substrate (waste activated sludge) and inoculum (anaerobic digested sludge from a digester fed with mixed activated and primary sludge) were collected from sewage treatment plants operated by Queensland Urban Utilities, Brisbane. The substrate was a single sample collected monthly, and it was representative of the WAS being fed into the anaerobic digesters at the sewage treatment plant. The substrate was diluted 50:50 with tap water to minimize clogging in the feed lines. Further details

on sample collection and storage are provided in Chapter 2, Section 2.2.1. The physico-chemical properties of substrate are shown in Table 4.1.

Table 4.1: Physico-chemical characteristic of waste activated sludge.

Parameter	Amount	Units	Parameter	Total	Soluble	Units
pH	6.2 ± 0.1	-	Al	114 ± 3	0.5 ± 0.5	mg L^{-1}
COD	22.9 ± 0.2	g L^{-1}	Ca	210 ± 5	44 ± 1	mg L^{-1}
TS	19.5 ± 0.6	g L^{-1}	Fe	131 ± 3	4 ± 0.3	mg L^{-1}
VS	14.0 ± 0.2	g L^{-1}	Mg	179 ± 5	105 ± 5	mg L^{-1}
NH_4-N	261 ± 5	mg L^{-1}	Na	493 ± 8	490 ± 5	mg L^{-1}
NOx-N	1 ± 0.05	mg L^{-1}	K	226 ± 3	206 ± 3	mg L^{-1}
			P	615 ± 9	354 ± 5	mg L^{-1}

Values are in mean ± 95% CI.

4.2.2 Reactors

A 1.5 L stainless steel reactor (SS 316), designed and developed locally with working volume of 0.75 L was used as high pressure anaerobic digester (HiPAD). Maximum working pressure of the reactor was 5.0 bar (gauge pressure) with a safe limit of 5.2 bar (6.2 absolute pressure). The reactor body was made by a 3 mm cylindrical shell, welded longitudinally. The top and bottom circumferences of the shell were welded with a 5 mm round plate, and flange respectively. All the connections and nozzles were made by SS 316 material, and welded on the reactor according to the design parameters. Further details on the pressure reactor are given in the Fig. 4.1. The temperature was controlled by means of tubes wrapped around the reactor, connected to a controlled temperature water bath at 37 ± 1°C. The reactor was equipped with a pressure transmitter with flush diaphragm (S-11, WIKA, Germany), an ultrasonic level senor (US06, GP Sand Sensor, China), a pressure relief valve (360046, SMC, Japan) and a mechanical pressure gauge (213.53, WIKA, Germany). All instruments were calibrated prior to use and were connected to a programmable logic control (PLC) unit (National Instruments, Australia) except the mechanical pressure gauge. Due to pressurized conditions, the pH sensor was installed in the effluent pipe just outside the reactor as shown in Fig. 4.2. A thermocouple type temperature sensor (WIKA, Germany) was also installed on the reactor to monitor the reactor temperature. Two peristaltic pumps with high pressure pump heads (Masterflex® L/S® 77250-62, Cole-Parmer, USA) were used for simultaneous feed and drain using a specialized tubing (L/S 16HP, 95664-16).

For the biogas release, the biogas outlet port was connected to a micro pressure regulator (upstream pressure: 15 bar, downstream pressure: minimum 20 mbar, AK1001S-4PL-44-00, SMC,

Japan). The outlet of the pressure regulator was connected to a solenoid valve (pressure rage: 0-15 bar, VX2220G-02-5D1, SMC, Japan). The solenoid valve was normally closed and was digitally operated by the PLC when the pressure inside the reactor exceeds the set limit. The biogas production was measured by custom made bucket type water filled gas meters (2.0-2.5 mL-biogas per bucket) connected to the solenoid value. All the data was logged on PLC. A schematic of the experiment and assembly is shown in Fig. 4.1 and 4.2 respectively. A detailed mechanical design of this high pressure anaerobic reactor is provide in Appendix 4.1.

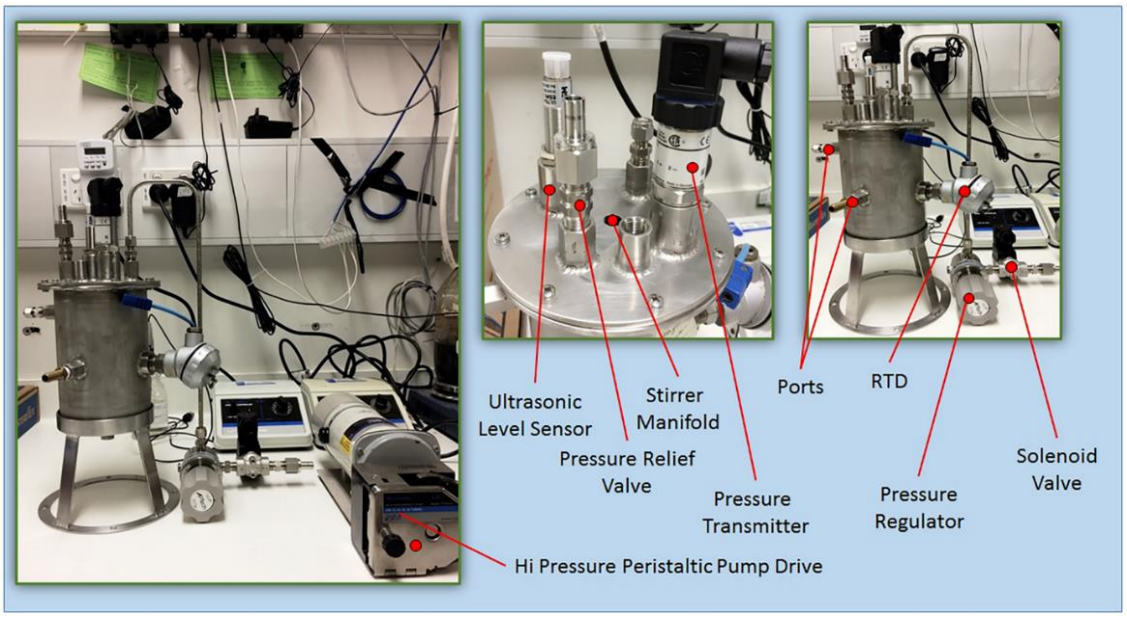

Figure 4.1: Assembly of the stainless steel reactor used for high pressure anaerobic digestion.

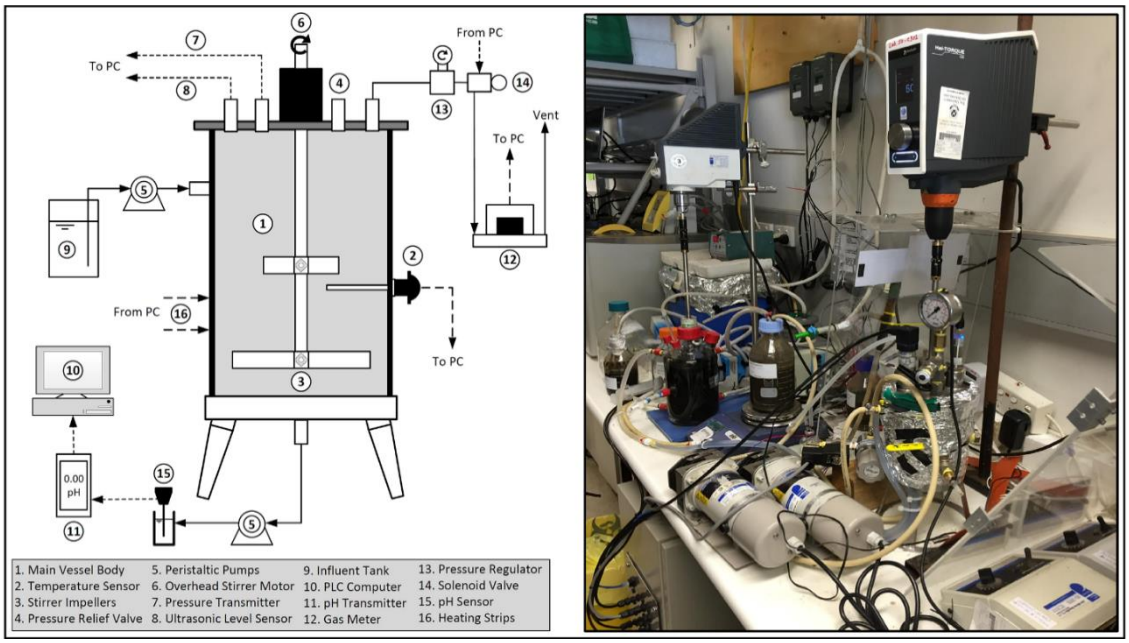

Figure 4.2: Schematic and actual experimental setup of high pressure anaerobic digestion.

A 1.5 L jacketed glass reactor was used as control and was connected using Masterflex® L/S® peristaltic pumps (model 7553-89, Cole-Palmer, USA) with multi-heads for simultaneous feed and drain. The process was controlled at 37 ± 1 °C by supplying heated water through the jacket of the reactor. The reactor pH was monitored and recorded in PLC supplied by Direct Automation, Australia. Biogas production was measured by custom made bucket type water filled gas meters connected to PLC. Further details are provided in Chapter 3.

4.2.3 Reactor Operation

The control and HiPAD were seeded with 750 mL of inoculum (14.82 gVS added) having COD:VS ratio of 1.62 (w/w basis). The pH of the assay was adjusted to 7.0. Each reactor was purged with nitrogen (N_2) gas at 5 L min^{-1} flow rate for at least two minutes to remove any excessive gas from the headspace and to provide anaerobic conditions. The reactors were operated at 12 day HRT, at an OLR of 1.9 ± 0.03 gCOD L^{-1} d^{-1} and a flow rate of 63 ± 2 mL d^{-1}. The HiPAD was initially operated similar to the control conditions and was set to the required pressure once steady state conditions were achieved, i.e. the performance parameters of the HiPAD were similar to the control reactor. This was achieved within 45-50 days. Table 4.2 shows the operating parameters of the control and HiPAD reactors. Steady state data of last two HRTs (minimum 24 days) at each pressure was analyzed and compared with the control reactor. The sludge in the reactor was sampled for analyses three times a week shortly before the feed. Mixing was done manually twice a day. The reactor sludge was well mixed before the feed and sample collection.

Table 4.2: Operating parameters of the control and HiPAD reactors.

Condition*	Duration (days)	Feed (g-COD L^{-1} d^{-1})[2]	Gauge Pressure set-points (bar)		HRT (d)[2]
			Minimum	***Maximum***	
1 bar	180	1.9 ± 0.03	-	-	12
2 bar[1]	48		0.98	1.05	
4 bar[1]	46		2.98	3.05	
6 bar[1]	46		4.98	5.05	

* The pressure quoted in the text is the absolute pressure. A 1 bar absolute pressure is quoted as control that continued parallel to the test reactor.

[1] pressure was achieved by self-generative biogas and it took 35 days to reach the final set point.

[2] constant feed and HRT at all conditions.

4.2.4 Analytical methods

The sludge samples were analyzed for Total solids (TS), VS, and COD three times a week, for SCOD and VFA twice a week, and for soluble and total elemental concentrations once a week. The sludge samples were collected for pyrosequencing during steady state conditions, at the last day of 2 and 6 bar pressures. Further details of the physico-chemical and molecular methods are provided in Chapter 2 Section 2.2.

4.2.5 Modelling and statistical analysis

All statistical analysis, and modelling, including generation of error bars based on two-tailed t-tests, linear modelling, and modelling of BMPs was done as described in Chapter 2.

Since the control and high pressure anaerobic reactors were mixed manually, variation in solids removal was expected. Therefore, the volatile solids (VS) destruction was calcualted by two methods; (1) Van Kleeck (VK) equation (Eq. 4.1) (Switzenbaum, Farrell et al. 2003), and (2) mass balance (MB) equation (Eq. 4.2) according to the Standard Methods described in (Ho 2014). The MB equation is the general method based on differences in the feed and effluent VS, while the VK assumes the amount of mineral solids is conserved during digestion, and uses the volatile fractions (VS/TS – $VS_{fraction}$) in the feed and effluent as a base referece.

$$VS\ destrucion\ (\%) = \frac{VS_{fraction\ (f)} - VS_{fraction\ (e)}}{VS_{fraction\ (f)} - (VS_{fraction\ (f)} \times VS_{fraction\ (e)})} \quad \text{(Eq. 4.1)}$$

Where $VS_{fraction\ (f)}$ and $VS_{fraction\ (e)}$ are the volatile fraction (VS/TS) in the feed and effluent solids respectively.

The mass balance equation practices the total VS concentration in the feed and effluent, as shown in Eq. 4.2.

$$VS\ destrucion\ (\%) = \frac{VS_{total\ (f)} - VS_{total\ (e)}}{VS_{total\ (f)}} \times 100 \quad \text{(Eq. 4.2)}$$

Where $VS_{total\ (f)}$ and $VS_{total\ (e)}$ are the total VS concentrations in mg L^{-1} of feed and effluent respectively.

4.3 Results and discussion

4.3.1 Effect of high pressure on methane production and soluble organics

The methane production at control and high pressure conditions is illustrated in Fig. 4.3. The methane production in the control reactor was variable due to the seasonal variations (refer to Chapter 2). The average methane yield in the control reactor were 42.3 ± 4.4, 27.3 ± 1.3 and 32.3 ± 1.9 L-CH_4 kg-VS_{fed}^{-1} during the period when the test reactor was running at 2, 4 and 6 bar respectively (Fig. 4.3 b). The average methane yield was 66.8 ± 3.6, 57.3 ± 3.2 and 58.5 ± 3.5 L-CH_4 kg-VS_{fed}^{-1} at 2, 4 and 6 bar respectively (Fig. 4.3 a). Methane yield for the test reactor was independent of the pressure but higher than the control. Similar effect of pressure on methane yield was reported in previous studies (Chen, Rößler et al. 2014). In this study, the methane contents in the biogas were 74.4 ± 0.01, 78.3 ± 0.01 and 81.4 ± 0.01% at 2, 4 and 6 bar respectively, these values were higher than the earlier findings as 71 ± 3 and 73 ± 3% methane at 3 and 8.9 bar respectively (Chen, Rößler et al. 2014).

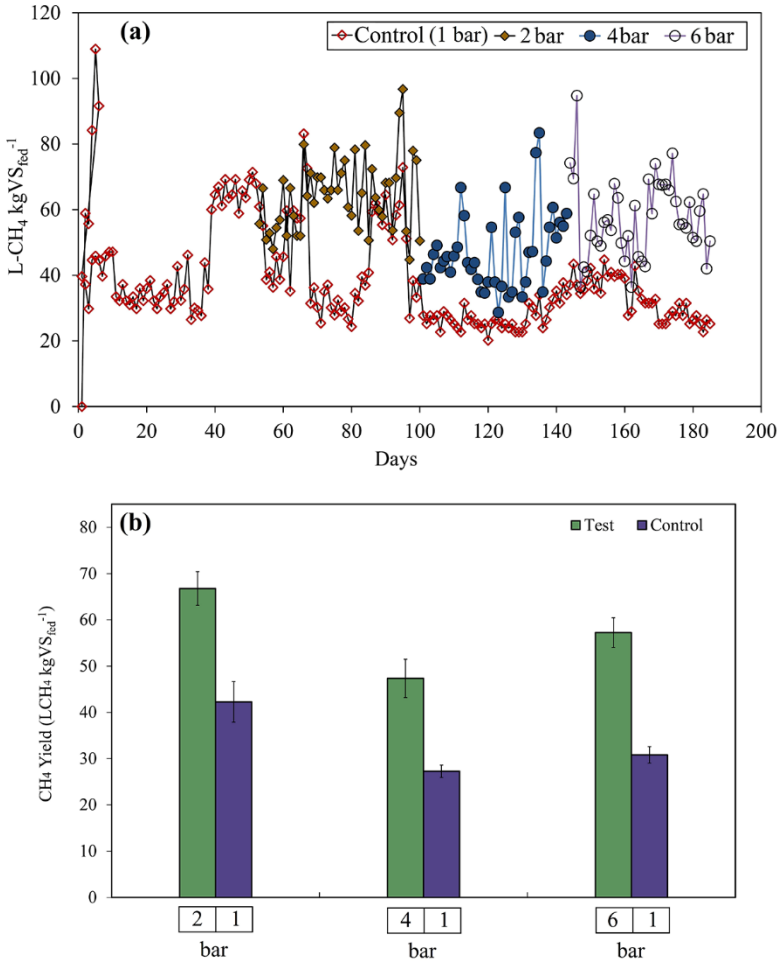

Figure 4.3: Variation in methane yield with pressure during anaerobic digestion; (a) continuous methane yield over the course of experiment at control and different pressure conditions, (b) average methane yield at different pressures in comparison to the control. Error bars represent the 95% confidence interval of the mean (n = 30).

The total VFA concentration at all pressure conditions and control was below 12 mg L^{-1}. No accumulation of VFA was observed at 6 bar and pH 6.4, similar results were observed during the low pH studies (Chapter 2 and 3). The SCOD concentration at all pressure conditions was always lower than the control that also shows the system stability at high pressure (Table 4.3). The VS and COD removals were decreased by approximately 5% at high pressures compared to the control (Fig. 4.4). A reduction in the solids removal at high pressure was expected as the reactor contents were mixed manually twice a day that could have influence the analyses.

Table 4.3: Effect of pressure on biogas production, COD removal and volatile solids destruction.

Absolute pressure (bar)	Total VFA (mg L^{-1})	SCOD (mg L^{-1})
1 (control)	11.7 ± 2.2	372 ± 19
2	11.9 ± 3.2	300 ± 21
4	6.5 ± 1.4	227 ± 37
6	11.7 ± 3.2	229 ± 26

Values are in mean ± 95% CI.

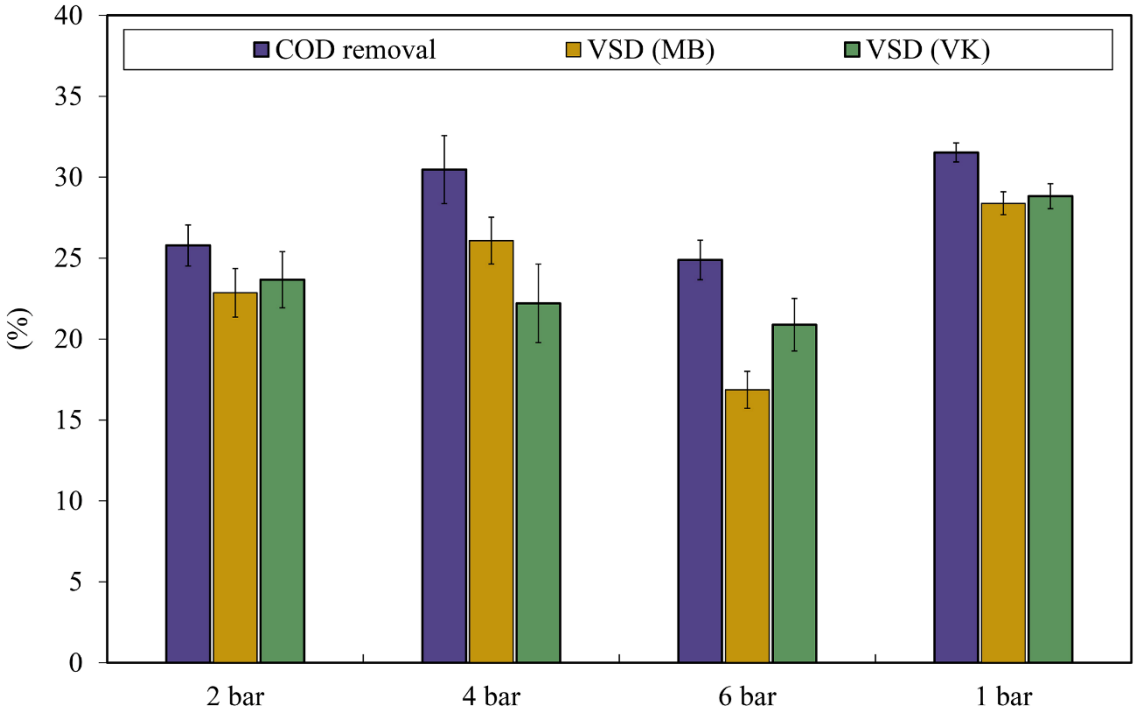

Figure 4.4: Chemical oxygen demand removal, volatile solids destruction (mass balance), and volatile solids destruction (Van Kleeck) at different pressure conditions. 1 bar pressure represents the control reactor.

4.3.2 Effect of high pressure on pH and carbon dioxide in biogas

Figure 4.5 shows that the pH was reduced from 7.05 to 6.72 at 2 bar, 6.56 at 4 bar, and then to 6.38 at 6 bar absolute pressure. The decrease in pH was expected and comparable with the previous findings of Chen, Rößler et al. (2014) who reported a final pH of 6.5 at 8.9 bar. The decrease in pH was due to increased solubility of CO_2 at high pressure (Chen, Rößler et al. 2014), and it caused a reduction in the CO_2 contents in the reactor headspace at high pressure. The average CO_2 content in biogas at control, 2 bar, 4 bar and 6 bar were found as 27.6, 19.8, 15.6 and 13.5 respectively.

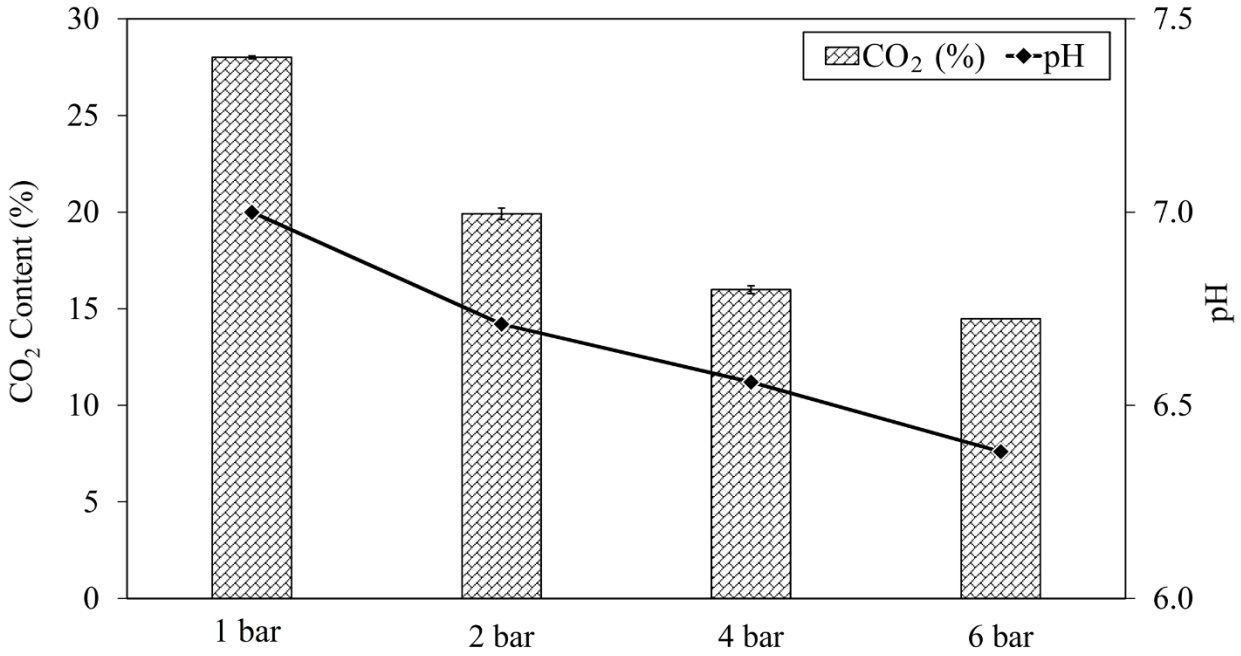

Figure 4.5: Effect of pressure on carbon dioxide contents in biogas and pH of the reactor sludge during anaerobic digestion.

4.3.3 Effect of high pressure on nutrients

The PO_4 concentration at control, 2 and 4 bar were 52, 56 and 65% of total P respectively, but it was remarkably increased to 75% at 6 bar. It was determined as 314 ± 0.2, 367 ± 0.3, 426 ± 0.4 and 490 ± 0.3 at control, 2 bar, 4 bar and 6 bar respectively (Fig. 4.6). High phosphate concentration at 6 bar was not only due to drop in pH (as reported in previous Chapters) but possible due to influence of high pressure on solubility of P complexes. The Mg release at 6 bar was almost double (54% of total) than in the control reactor (22% of total). This shows Mg-P complexes such as struvite were dissociated at the high pressure conditions. These results demonstrate that there is a potential to recover key nutrients by solubilizing them at high pressure during AD. The change in the ammonium concentration with pressure is shown in Fig. 4.7. The ammonium concentration in the test reactor was

lower than control at all test conditions, but there was no influence of change in pressure on ammonium concentration. Increased concentration of ammonium was expected with increase in pressure due to possible dissociation of struvite (refer to Chapter 2 section 2.3.3).

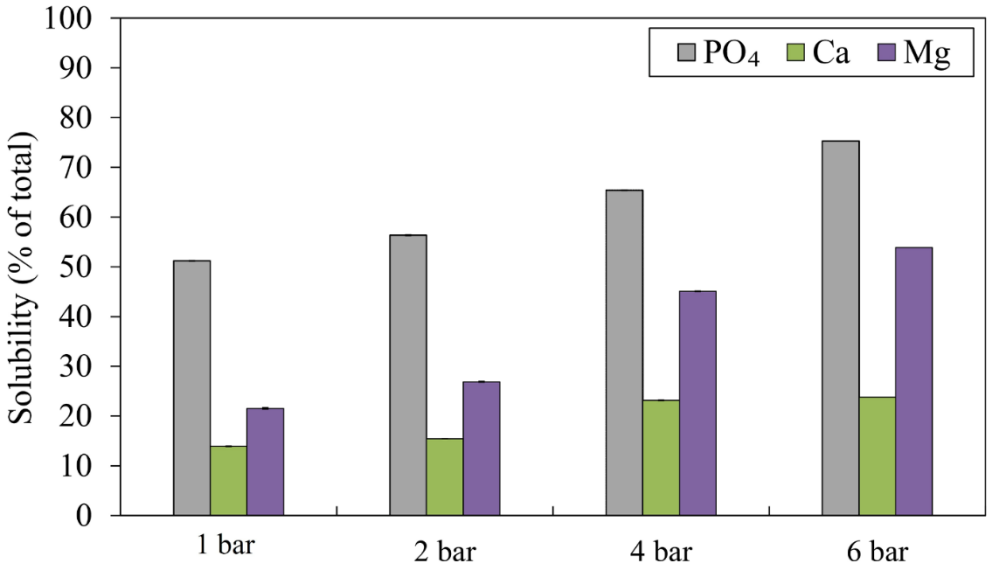

Figure 4.6: Effect of pressure on phosphate and cations solubility during anaerobic digestion. Error bars represent the 95% confidence interval of the mean (n = 4).

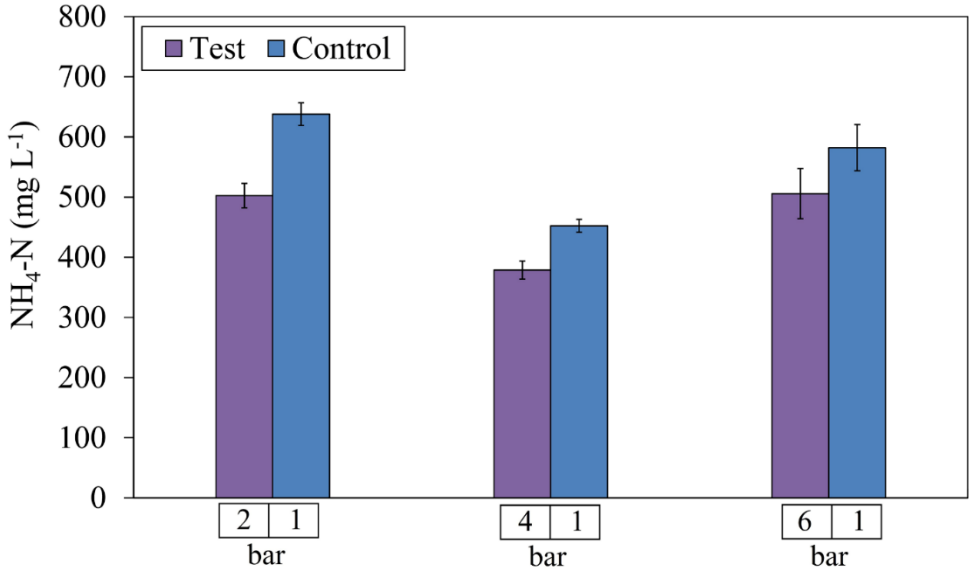

Figure 4.7: Variation on ammonium concentration at different pressure conditions in comparison with control. Error bars represent the 95% confidence interval of the mean (n = 4).

4.3.4 Effect of pressure on microbial community

The effect of pressure on microbial community was evaluated through PCA (Fig. 4.8) and abundance (Fig. 4.9) plots. Only 1 bar, 2 bar, and 6 bar are shown, as the 4 bar samples could not be analyzed in time for thesis submission. In Fig. 4.7a, the principal component analysis (PCA) revealed that PC1 and PC2 occupied 85% of the total variance, with both factors at comparable levels, and a fairly even distribution between the major samples. Both archaeal and bacterial communities responded to increasing levels of pressure. The feed sample had bacteria up to 99% of the OTU and minimal archaeal community as expected (mainly class Methanomicrobia). Methanosaeta were stable across all pressures, but the remaining archaeal community developed substantially as pressure increased. At 2 bar an organism belonging to class DSEG (Deep Sea Euryarchaeotic Group-a marine microbe) emerged, together with Methanocorpusculum as other major archaea (along with Methanosaeta). The presence of DSEG at high pressure seemed to be due to high pressure only as it has been previously reported to be available in marine sediments where, a high pressure on sediments is exerted by the height of the water column (Aoki, Ehara et al. 2014). This shows that by increasing the pressure from 1 to 2 bar, the archaeal community influenced and it seemed to be shifted towards class DSEG. In addition, a minor amount of an unclassified microbe genes Methanocella (1% of total archaea) within the class Methanomicrobia was also observed, which was not found in the control sample (Fig. 4.9). The Methanocella was significantly increased at 6 bar (48% of total archaea) as shown in Fig. 4.9. However, the microbial community at 6 bar was still dominated by bacteria (90% of OTU), with a shift towards Bacteroidetes. The phylum Bacteroidetes are proteolytic bacteria and are responsible to metabolize amino acids to produce VFA, therefore, these were available at each pressure conditions including control, with a significant increase at 6 bar. However, no VFA accumulation was observed at 6 bar suggesting that VFA degradation was not impacted.

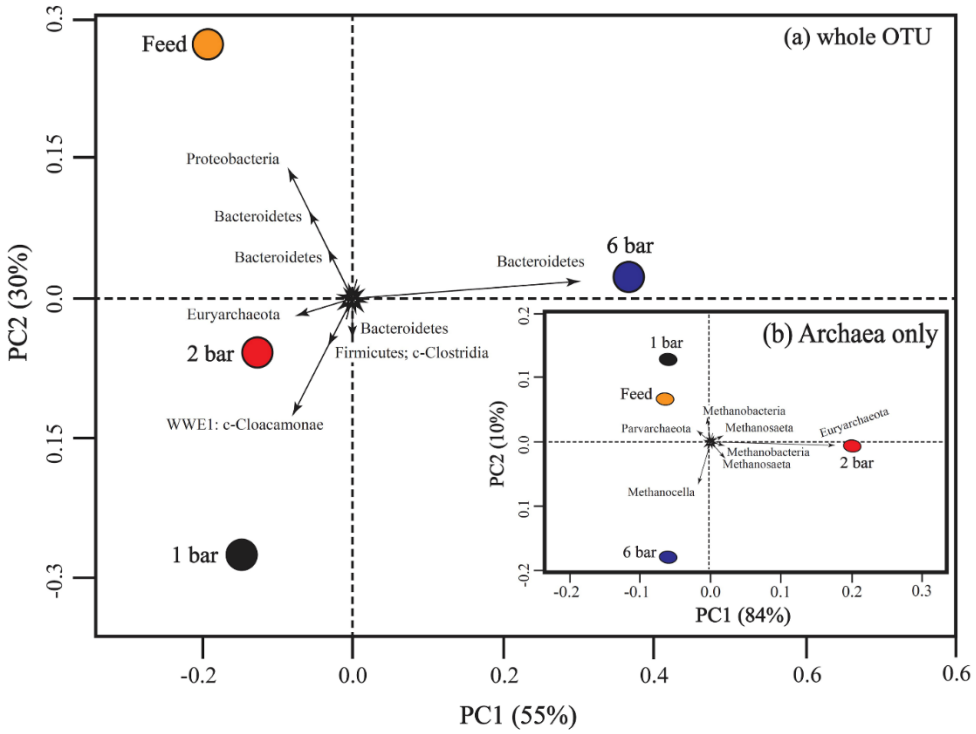

Figure 4.8: Principal component analysis biplot, (a) whole operational taxonomic unit, (b) archaea only. Colored circles and eclipses represent the sample of which DNA was extracted. Arrows show the microbial community in the biplot region.

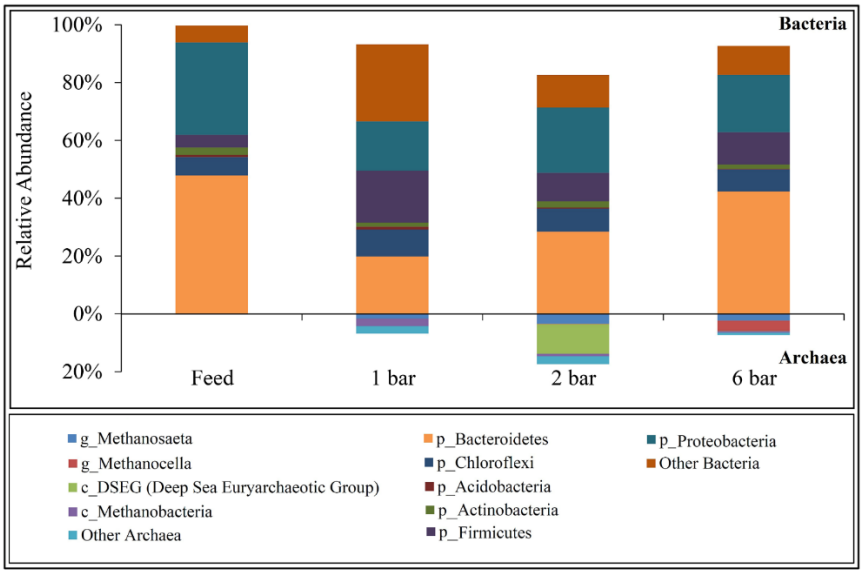

Figure 4.9: Relative abundance of bacteria and archaea in the feed, control and different pressure conditions during anaerobic digestion. Archaeal and bacterial communities differentiate each other by axis.

4.3.5 Conclusions

It was found that 2, 4, and 6 bar absolute pressures solubilized P up to 56.3 ± 0.05, 65.4 ± 0.1, and 75.3 ± 0.05% of its total concentration respectively. The Mg was released linearly with the increasing

pressure ($p = 0.01$), but Ca concentration was unchanged. The specific methane yield at all pressure conditions was increased compared to the control. However, VSD and COD removal were not significantly affected, indicating no substantial difference in operational outcomes. The soluble organics such as VFAs and SCOD at all pressure conditions were either similar or less than the control reactor. Molecular analysis revealed that the abundance of methanogens was about 10% of total OTU in the control and 6 bar samples, whereas it was 20% at 2 bar. It was identified that there was a shift in the microbial community especially with the archaea, which seemed to be shifted towards DSEG and Methanocella at 2 and 6 bar absolute pressure respectively. The application of high pressure up to 6 absolute pressure could be possible in the current anaerobic digester at a treatment facility, but this would require design modifications, and increase capital costs. As phosphorous pricing increases however, this will enable economic recovery without chemical addition, and an increase in usable methane.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The work in this thesis has achieved its primary objective of increasing in-reactor P availability during anaerobic digestion, to improve recovery yield of P following anaerobic digestion and reduce in-reactor precipitation. To achieve this, three techniques were tested during AD: low pH, high pressure, and chemical additives. The low pH AD was tested in both batch and continuous mode to access feasibility of the technique for commercial purposes, and acid dosing rates were measured at each pH. While the high pressure and chemical additives were only tested in continuous and batch mode, respectively. Based on the research objectives, and following key conclusions were derived.

Research Objective 1 and 2 (Influence of low pH on P solubility during anaerobic digestion):

- *Influence on PO_4, Ca and Mg.* PO_4, Ca and Mg concentration increased proportionally as the pH reduced from 7.0 to 5.5. In-reactor PO_4 was found to be >75% of its total concentration during batch and continuous AD at pH 5.5, which was due to the dissociation of inorganic P-precipitants such as Ca-P and Mg-P. The dissociation of these complexes at low pH solubilized Ca and Mg up to 50 and 90% of their total concentrations respectively.
- *Influence on methane production, VFA and SCOD:* The methane yield was decreased by 33% at pH 5.25 compared to the control during batch AD, and 50% at 5.5 compared to the control during continuous AD. In both experiments, CO_2 in the biogas was increased at low pH and was found up to 45% at pH 5.0-5.5. A decrease in methane production caused an increase in soluble organics, with the propionic acid being dominant at low pH. The propionic acid was found up to 78% of total VFAs at pH < 6. Overall, the total VFAs were respectively found as 300 and 430 mg L^{-1} in batch and continuous AD at pH 5.25-5.0. Similarly, the soluble COD was also increase at low pH, it was respectively found as 1300 and 2100 mg L^{-1} in batch and continuous AD respectively. Regarding acid requirements to maintain low pH, approximately 0.6, 1.0, 2.4, and 3.0 mL-1 M HCl was required per litre of sludge to maintain pH 6.5, 6.0, 5.5, and 5.0 respectively.
- *Solids removal.* Since soluble organics were increased at low pH conditions, these caused an accumulation of undegraded organic matter that could not degrade. The accumulation of undegraded organic matter resulted in a lower VS destruction at low pH. The VS destruction at control and pH 5.5 was measured as 32 and 9% respectively. The neutral and low pH digestates were further subjected to batch AD. It was found that the undegraded organic matter

was further degraded in the BMP mixed assays of control and low pH digestates, revealing total VS destruction up to 39%, similar to the control.

- *Microbial community.* The acetoclastic community was not influenced by low pH during batch AD, while it was largely impacted by pH < 6 in the continuous AD. The Bacteria were dominant (84-89%) and archaea were 11-16% at pH 6.5 and pH 6.0 (having *Methanosaeta* as 6-9% of total community). The proportion of archaea was reduced to 5 and 3%, while *Methanosaeta* was reduced to 1 and 0.1%, at pH 5.5 and 5.0 respectively, showing that these two pH conditions have substantially influenced the methanogens. *Bacteroidetes* and *Chloroflexi* dominated the total bacterial community (79%) and was mainly due to the acidogens which could not convert the complex organics into methane.

Research Objective 3 (Influence of high pressure on P solubility during anaerobic digestion):

- *PO_4 and cations.* PO_4 was found as 56, 65, and 75% of its total concentration at 2, 4, and 6 bar absolute pressure respectively, while the control (1 bar absolute pressure) solubilized P as 51% of total. The Mg was released linearly with an increase in pressure, but Ca concentration was unchanged. The Mg was found in soluble form as 22, 27, 45 and 54% of its total concentration respectively at 1, 2, 4, and 6 bar conditions.
- *Methane production.* The methane yield at all pressure conditions was increased by approximately 40% compared to the control. An increase in methane yield was addressed because of high methane contents in biogas. The high pressure solubilized CO_2 in the reactor sludge, which mainly produced carbonic acid. The carbonic acid reduced the solution pH from 7.0 to 6.38 due to an increase in CO_2 partial pressure.
- *Soluble organics.* The soluble organics such as VFAs and SCOD at all pressure conditions were either similar or less than the control reactor.
- *Solids removals.* The mass balance VS destruction was reduced with increasing pressure, and were measure as 28, 22, 26, and 17% at 1, 2, 4, and 6 bar respectively. However, the Van Kleeck VS destruction was less influenced by pressure and was found as 29, 24, 22, and 21% at 1, 2, 4, and 6 bar conditions. The mass balance VSD is likely incorrect due to intermittent mixing.
- *Microbial community.* Molecular analysis revealed that the methanogen total abundance was increased with pressure. *Methanosaeta* was found at all pressure conditions including 1, 2 and 6 bar absolute pressures, but Methanocella was the only microbe that was not found at 1 bar. The Methanocella was increased by 96% at 6 bar compared to 2 bar.

5.2 Recommendations

The low-pH, high-pressure and chemical additives based single stage anaerobic digestion are novel processes for increasing P solubility during anaerobic digestion. There are number of potential directions for improving understanding or extending the application of the developed techniques. The work presented in this thesis provides an opportunity for further development of the techniques to improve P solubility. Of particular interest is an extension of the low pH lab-scale process developed in this thesis to a pilot-scale or full-scale process. There are a number of possible direction for future work that would both improve the general understanding of P precipitation during anaerobic digestion through plant-wide modelling and facilitate development of upstream technique to reduce P precipitation during AD. Following research and development activities are recommended based on this work:

- As shown in Chapter 2 and 3, reduced methane production was observed during low pH AD. There is a potential to reduce loss in methane by implementing a second stage AD following low pH AD. Further investigation is required to optimize this two-stage process to achieve maximum P recovery and methane (see Figure 5.1).

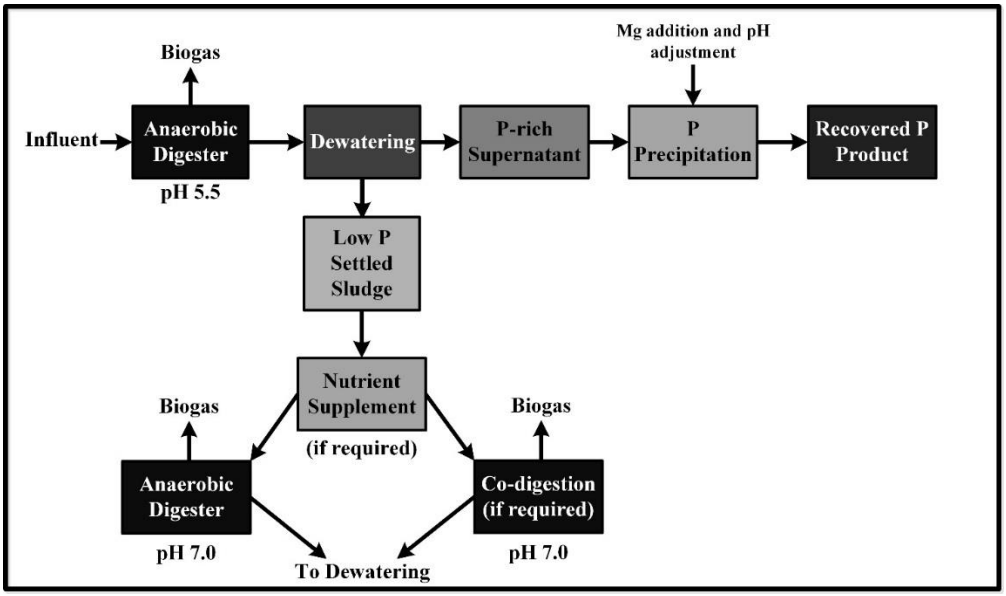

Figure 5.1: Proposed process flow diagram of two-stage anaerobic digestion with an intermediate phosphorus recovery system.

- Continuous acid requirement is likely the major operating cost during low pH AD. The cost can be reduced through co-digestion of the WAS with other agro-industrial waste streams that would naturally buffer the system below pH 6.0. However, this needs to be thoroughly tested at a bench scale, using methodology developed in this work.

- Since, acidogens were dominant at low pH, a detailed microbial analyses are required to identify relevant species responsible in the suppression of methanogens. The carbohydrates and proteins were not investigated in this thesis, but are needed to test during low pH operations that could provide useful information regarding the accumulation of soluble organics.
- As shown in Chapter 4, high pressure significantly increased methane production without influencing microbial community and soluble organics. Methane may be supersaturated (up to 60x) in the digester liquors (Pauss, Samson et al. 1990), but readily strips through dewatering equipment and has no detrimental impact, given belt filter presses operate through a pressing process (no settling), and centrifuges apply large amounts of shear, which readily strips gases. However, the only concern with high pressure AD is the capacity of an anaerobic digester to maintain a pressure up to 6 bar in the digester headspace. An existing digester is needed to be verified for given pressure ranges, while new digesters can be constructed in a high pressure context.

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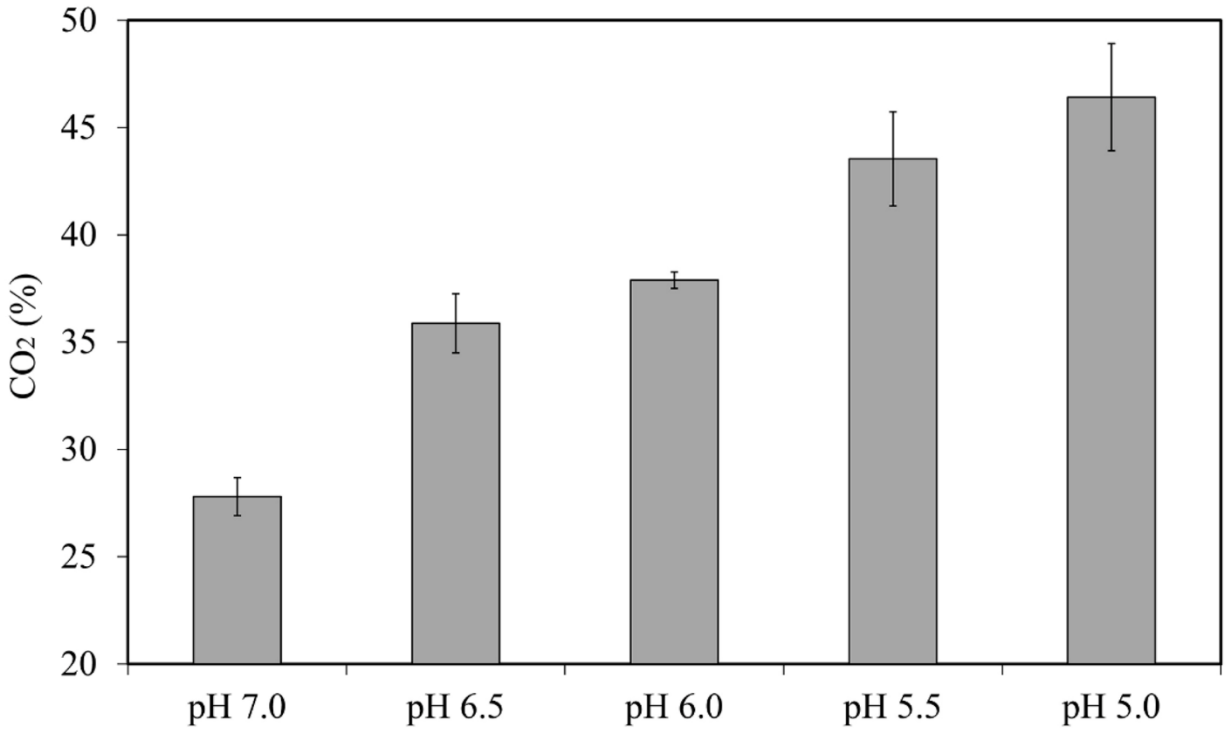
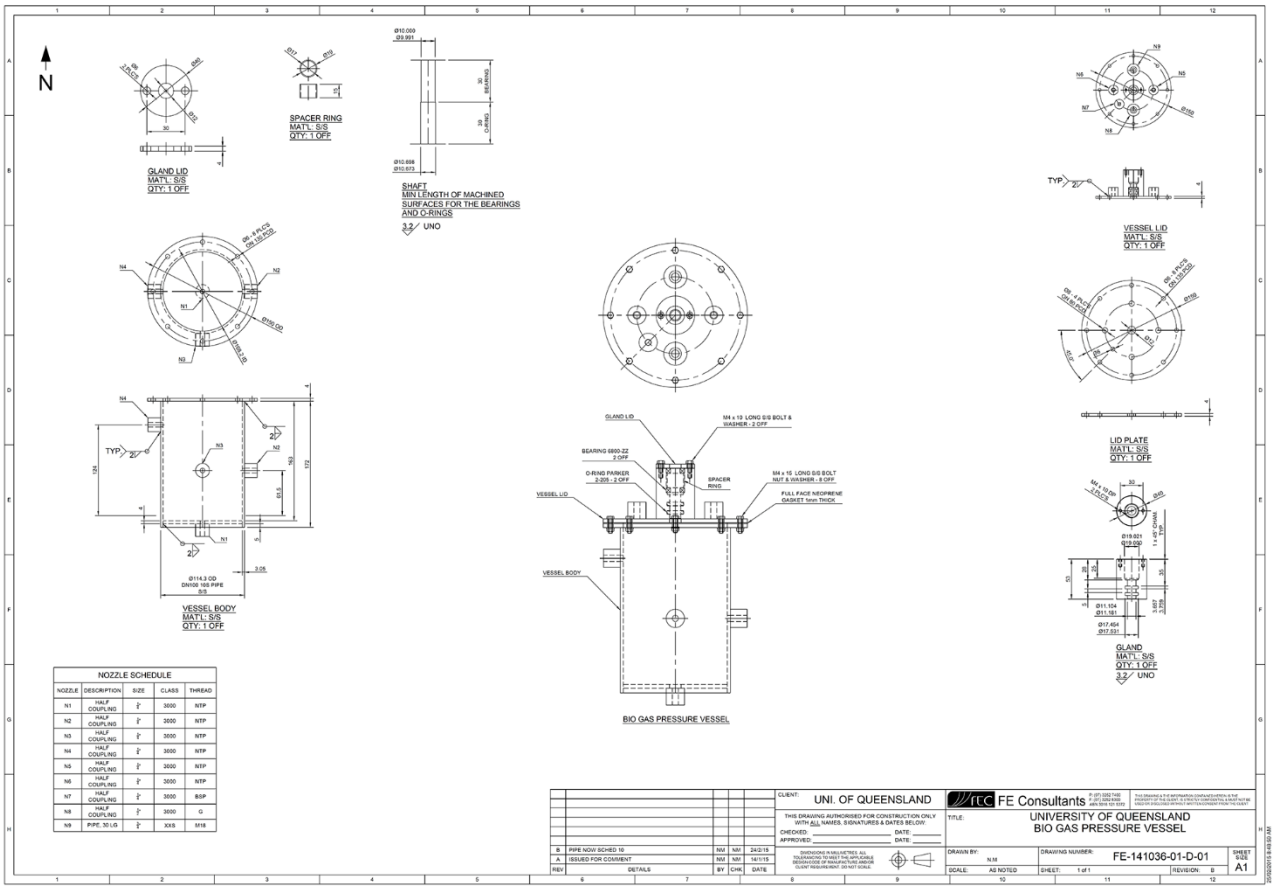
Appendices

Appendix 3.1



Appendix 3.1: Average CO_2 contents in biogas at each pH condition. The error bars show the standard error calculated at 95% confidence interval.

Appendix 4.1



Appendix 4.1: Detailed mechanical design of high pressure anaerobic reactor.